FITNESS ASSAY AND ASSOCIATED METHODS

TECHNICAL FIELD OF THE INVENTION

The present invention relates to a biochemical
5   fitness assay and related methods.

BACKGROUND OF THE INVENTION

The development of drug resistance is one of the
most perplexing challenges in the field of medicine. One
10   of the most common causes of drug failure in the
treatment of diseases involving replicating biological
entities, for example, cancer and infectious diseases, is
the emergence of drug resistance. One of the most
dramatic and tragic examples of drug resistance can be
15   found in connection with the antiviral therapy of
acquired immune deficiency syndrome (AIDS).

AIDS is a fatal disease, reported cases of which
have increased dramatically within the past several
years. Estimates of reported cases in the very near
20   future also continue to rise dramatically.

The AIDS virus was first identified in 1983. It has
been known by several names and acronyms. It is the
third known T-lymphocyte virus (HTLV-III), and it has the
capacity to replicate within cells of the immune system,
25   causing profound cell destruction. The AIDS virus is a
retrovirus, a virus that uses reverse transcriptase
during replication. This particular retrovirus is also
known as lymphadenopathy-associated virus (LAV), AIDS-
related virus (ARV) and, most recently, as human
30   immunodeficiency virus (HIV). Two distinct families of
HIV have been described to date, namely HIV-1 and HIV-2.

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The acronym HIV will be used herein to refer to HIV
viruses generically.

Specifically, HIV is known to exert a profound
cytopathic effect on the CD4+ helper/inducer T-cells,
5　　thereby severely compromising the immune system. HIV
infection also results in neurological deterioration and,
ultimately, in the death of the infected individual.

The field of viral chemotherapeutics has developed
in response to the need for agents effective against
10　　retroviruses, in particular HIV. For example anti-
retroviral agents, such as 3'-azido-2',3'-
dideoxythymidine (AZT), 2'3'-dideoxycytidine (ddC), and
2'3'-dideoxyinosine (ddI) are known to inhibit reverse
transcriptase. There also exist antiviral agents that
15　　inhibit transactivator protein. Nucleoside analogs, such
as AZT, are currently available for antiviral therapy.
Although very useful, the utility of AZT and related
compounds is limited by toxicity and insufficient
therapeutic indices for fully adequate therapy.

20　　Retroviral protease inhibitors also have been
identified as a class of anti-retroviral agents.
Retroviral protease processes polyprotein precursors into
viral structural proteins and replicative enzymes. This
processing is essential for the assembly and maturation
25　　of fully infectious virions. Accordingly, the design of
protease inhibitors remains an important therapeutic goal
in the treatment of AIDS.

The use of HIV protease inhibitors, in combination
with agents that have different antiretroviral mechanisms
30　　(e.g., AZT, ddI and ddT), also has been described. For
example, synergism against HIV-1 has been observed

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between certain C_2 symmetric HIV inhibitors and AZT
(Kageyama et al., *Antimicrob. Agents Chemother.*, 36, 926-
933 (1992)).

5   Numerous classes of potent peptidic inhibitors of
protease have been designed using the natural cleavage
site of the precursor polyproteins as a starting point.
These inhibitors typically are peptide substrate analogs
in which the scissile P_1-P_1' amide bond has been replaced
by a non-hydrolyzable isostere with tetrahedral geometry
10   (Moore et al, *Perspect. Drug Dis. Design*, 1, 85 (1993);
Tomasselli et al., *Int. J. Chem. Biotechnology*, 6 (1991);
Huff, *J. Med. Chem.*, 34, 2305 (1991); Norbeck et al.,
Ann. Reports Med. Chem., 26, 141 (1991); and Meek, *J.
Enzyme Inhibition*, 6, 65 (1992)). Although these
15   inhibitors are effective in preventing the retroviral
protease from functioning, the inhibitors suffer from
some distinct disadvantages. Generally, peptidomimetics
often make poor drugs, due to their potential adverse
pharmacological properties, i.e., poor oral absorption,
20   poor stability and rapid metabolism (Plattner et al, *Drug
Discovery Technologies*, Clark et al., eds., Ellish
Horwood, Chichester, England (1990)).

The design of the HIV-1 protease inhibitors based on
the transition state mimetic concept has led to the
25   generation of a variety of peptide analogs highly active
against viral replication *in vitro* (Erickson et al,
Science, 249, 527-533 (1990); Kramer et al., *Science*,
231, 1580-1584 (1986); McQuade et al., *Science*, 247, 454-
456 (1990); Meek et al., *Nature (London)*, 343, 90-92
30   (1990); and Roberts et al., *Science*, 248, 358-361
(1990)). These active agents contain a non-hydrolyzable,

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dipeptidic isostere, such as hydroxyethylene (McQuade et
al., *supra*; Meek et al., *Nature (London)*, 343, 90-92
(1990); and Vacca et al., *J. Med. Chem.*, 34, 1225-1228
(1991)) or hydroxyethylamine (Ghosh et al., *Bioorg. Med.*
5   *Chem. Lett.*, 8, 687-690 (1998); Ghosh et al., *J. Med.*
Chem., 36, 292-295 (1993)); Rich et al., *J. Med. Chem.*,
33, 1285-1288 (1990); and Roberts et al., *Science*, 248,
358-361 (1990)) as an active moiety that mimics the
putative transition state of the aspartic protease-
10 catalyzed reaction.

Two-fold (C$_2$) symmetric inhibitors of HIV protease
represent another class of potent HIV protease
inhibitors, which were created by Erickson et al., on the
basis of the three-dimensional symmetry of the enzyme
15 active site (Erickson et al. (1990), *supra*). Typically,
however, the usefulness of currently available HIV
protease inhibitors in the treatment of AIDS has been
limited by relatively short plasma half-life, poor oral
bioavailability, and the technical difficulty of scale-up
20 synthesis (Meek et al. (1992), *supra*).

In a continuing effort to address the problem of
short plasma half-life and poor bioavailability, new HIV
protease inhibitors have been identified. For example,
HIV protease inhibitors incorporating the 2,5-diamino-
25 3,4-disubstituted-1,6-diphenylhexane isostere are
described in Ghosh et al., *Bioorg. Med. Chem. Lett.*, 8,
687-690 (1998) and U.S. Patent Nos. 5,728,718 (Randad et
al.). HIV protease inhibitors, which incorporate the
hydroxyethylamine isostere, are described in U.S. Patent
30 Nos. 5,502,060 (Thompson et al.), 5,703,076 (Talley et
al.), and 5,475,027 (Talley et al.).

Recent studies, however, have revealed the emergence

of mutant strains of HIV, in which the protease is

resistant to the C_2 symmetric inhibitors (Otto et al.,

PNAS USA, 90, 7543 (1993); Ho et al., *J. Virology*, 68,

5   2016-2020 (1994); and Kaplan et al., *PNAS USA*, 91, 5597-

5601 (1994)).   In one study, the most abundant mutation

found in response to a C_2 symmetry based inhibitor was

Arg to Gln at position 8 (R8Q), which strongly affects

the $S_3/S_{3'}$ subsite of the protease binding domain.   In

10   this study, the shortening of the $P_3/P_{3'}$ residues resulted

in inhibitors that were equipotent towards both wild-type

and R8Q mutant proteases (Majer et al., 13th *American

Peptide Symposium*, Edmonton, Canada (1993)).   Inhibitors

have been truncated to $P_2/P_{2'}$ without significant loss of

15   activity (Lyle et al., *J. Med. Chem.*, 34, 1230 (1991);

and Bone et al., *J. Am. Chem. Soc.*, 113, 9382 (1991)).

These results suggest that inhibitors can be truncated

and yet maintain the crucial interactions necessary for

strong binding.   The benefits of such an approach include

20   the elimination of two or more peptide bonds, the

reduction of molecular weight, and the diminishment of

the potential for recognition by degradative enzymes.

More recently, new mutant strains of HIV have

emerged that are resistant to multiple, structurally

25   diverse, experimental and chemotherapeutic retroviral

protease inhibitors.   Such multidrug-resistant HIV

strains are typically found in infected patients, who had

undergone treatment with a combination of HIV protease

inhibitors or a series of different HIV protease

30   inhibitors.   The number of reported cases of patients

infected with multidrug-resistant HIV is rising

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dramatically. Tragically for these patients, the
available options for AIDS chemotherapy and/or HIV
management is severely limited or is, otherwise,
completely nonexistent.

5   Drug resistance is unfortunately the most common
reason for drug failures generally. One of the most
dramatic examples of drug failure due to resistance is in
HIV therapy. Once HIV resistance is obtained to first-
line therapy, the chances of future success are greatly

10   diminished because of the development of multidrug cross
resistance. Other diseases involving infectious agents
(e.g., viruses, bacteria, protozoa, and prions) or other
disease-causing cells (e.g., tumor cells) present similar
challenges in that drug resistance is a primary cause of

15   drug failure.

In view of the foregoing problems, there exists a
need to determine whether a mutant will be capable of
replicating in the presence of a drug. There also exists
a need for a method of predicting whether drug resistance

20   is likely to emerge in a disease involving a replicating
biological entity. There is also a need for a method of
devising a long-term therapeutic regimen that minimizes
the likelihood that resistance will occur in a disease
involving a replicating biological entity. Moreover,

25   there is a need for a method of preventing or inhibiting
the development of drug resistance in such diseases.

The present invention provides such methods. These
and other advantages of the present invention, as well as
additional inventive features, will be apparent from the

30   description of the invention provided herein.

BRIEF SUMMARY OF THE INVENTION

The present invention is predicated on the surprising and unexpected discovery that biochemical "vitality," as described below, can be used to determine the biological fitness of a mutant replicating biological entity relative to its predecessor under the selection pressure of an inhibitor. The present invention provides an assay for determining the biochemical fitness of a biochemical target (i.e., a biomolecule having a biochemical function), of a mutant replicating biological entity relative to its predecessor's biochemical target, in the presence of a compound that acts upon the biochemical target. The assay method of the present invention includes obtaining the predecessor, determining the biochemical vitality of the biochemical target of both the predecessor and the mutant in the presence of a compound that acts upon the biochemical target of the predecessor, and comparing the vitality of the mutant's biochemical target relative to the vitality of the predecessor's biochemical target. Where the biochemical vitality of the mutant is greater than the biochemical fitness of the predecessor, the mutant is predicted to be more biologically fit in the presence of the compound. The assay method can thus be used to predict the emergence of drug resistance for a particular replicating biological entity (e.g., a disease-causing cell) in the presence a drug (e.g., an inhibitor). Utilization of the assay in accordance with the present invention permits the administration of an inhibitor or combination of

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inhibitors to treat a disease in a way that decreases the
likelihood that drug resistance will develop.

The present invention further provides a continuous
fluorogenic assay for measuring the anti-HIV protease
5   activity of a protease inhibitor. The continuous
fluorogenic assay of the present invention utilizes a
substrate of the formula Ala-Arg-Val-Tyr-Phe(NO$_2$)-Glu-
Ala-Nle-NH$_2$. The continuous fluorogenic assay of the
present invention is highly sensitive and particularly
10   useful for the prediction of the antiviral inhibitory
activity of a compound against mutant HIV.

The present invention further provides a method of
administering a therapeutic compound that inhibits a
biochemical target of a disease-causing replicating
15   biological entity. The therapeutic compound, when
administered in accordance with the method of the present
invention, minimizes the chances that the disease-causing
entity will develop drug resistance. As such, the method
of administering a therapeutic compound in accordance
20   with the present invention improves the chances of long-
term success in therapy.

The present method of administering a therapeutic
compound involves the identification of at least one
mutant replicating biological entity (the mutant) capable
25   of evolving from the disease-causing replicating
biological entity (the predecessor). Biochemical fitness
is determined by comparing the biochemical vitality of
the mutant's biochemical target with the biochemical
vitality of the predecessor's biochemical target.
30   Biochemical fitness is determined in the presence of a
drug (e.g, an inhibitor). The biochemical vitality of

the mutant's biochemical target is compared to
biochemical vitality of the predecessor's biochemical
target in the presence of the drug. When there are two
or more drugs available for treatment, biochemical

5   fitness can be determined for each drug in accordance
with the present invention. A therapeutic compound is
then administered from among one of the compounds that
produces a lower value for biochemical fitness with
respect to one or more mutants. Administration of a

10 therapeutic compound producing a lower fitness value for
a particular mutant indicates that the predecessor is
less likely to develop resistance in the presence of that
compound.

The present invention also provides a method of

15 preventing the development of drug resistance of HIV in an
HIV-infected mammal by the administration of a drug
resistance-inhibiting effective amount of a compound of
the formula:
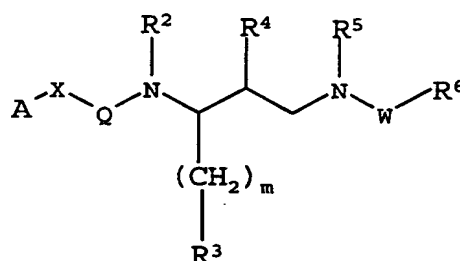
$$A-X-Q-N(R^2)-CH((CH_2)_m-R^3)-CH(R^4)-CH_2-N(R^5)-W-R^6$$

(I),

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or a pharmaceutically acceptable salt, a prodrug, or an
ester thereof, or a pharmaceutical composition thereof,
wherein:

A is a group of the formula:
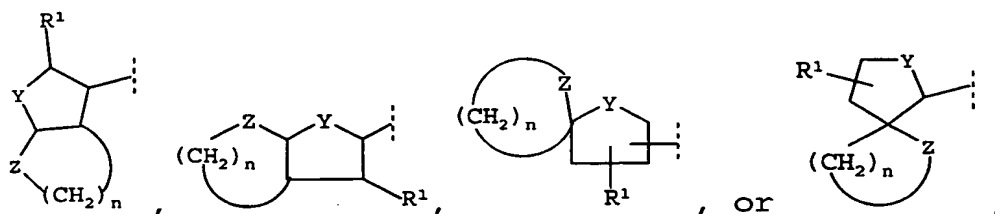
R^1 is H or an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a cycloalkylalkyl, an aryl, an aralkyl, a heterocycloalkyl, a heterocycloalkylalkyl, a heteroaryl, or a heteroaralkyl radical, which unsubstituted or substituted;

Y and Z are the same or different and are each selected from the group consisting of CH_2, O, S, SO, SO_2, NR^8, R^{8}C(O)N, R^{8}C(S)N, R^{8}OC(O)N, R^{8}OC(S)N, R^{8}SC(O)N, R^{8}R^{9}NC(O)N, and R^{8}R^{9}NC(S)N, wherein R^8 and R^9 are each H, an alkyl, an alkenyl, or an alkynyl;

n is an integer from 1 to 5;

X is a covalent bond, CHR^{10}, CHR^{10}CH$_2$, CH$_2$CHR^{10}, O, NR^{10}, or S, wherein R^{10} is H, an alkyl, an alkenyl, or an alkynyl;

Q is C(O), C(S), or SO_2;

R^2 is H, an alkyl, an alkenyl, or an alkynyl;

m is an integer from 0 to 6;

R^3 is a cycloalkyl, a heterocycloalkyl, an aryl, or a heteroaryl which is unsubstituted or substituted;

R^4 is OH, =O (keto), NH$_2$, or a derivative thereof;

R^5 is H, a C_1-C_6 alkyl radical, a C_2-C_6 alkenyl radical, or (CH$_2$)$_q$R^{14}, wherein q is an integer form 0 to 5, and R^{14} is a cycloalkyl, a heterocycloalkyl, an aryl, or a heteroaryl which is unsubstituted or substituted;

W is C(O), C(S), S(O), or SO_2; and

R^6 is a cycloalkyl, heterocycloalkyl, aryl, or heteroaryl which is unsubstituted or substituted.

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Optionally, R^5 and R^6, together with the N-W bond of formula (I), comprise a macrocyclic ring which can contain at least one additional heteroatom in the ring skeleton.

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BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 illustrates the synthesis of a particular sulfonamide isostere core of a compound of the present invention.

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Figure 2 illustrates the synthesis of a bis-tetrahydrofuran ligand and the optical resolution thereof.

15   Fig. 3A illustrates the synthesis of a compound of the present invention via coupling of a bis-tetrahydrofuran ligand to a sulfonamide isostere of the present invention.

20   Fig. 3B illustrates the synthesis of a compound of the present invention via coupling of a bis-tetrahydrofuran ligand to a sulfonamide isostere of the present invention.

25   Figure 4 illustrates generally the present method of synthesizing a compound of the present invention.

Figures 5A-5D illustrate the structures of particular compounds that were tested against various
30   drug resistant HIV mutants.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention is predicated on the surprising and unexpected discovery to that the "vitality" of a biochemical target of a mutant replicating biological entity relative to that of its predecessor's biochemical target can be used to predict the biological fitness of the mutant under the selection pressure of an inhibitor of the biochemical target. The "vitality" of a biochemical target of a mutant replicating biological entity relative to the "vitality" of its predecessor's biochemical target is defined herein as the "biochemical fitness."

"Vitality" as utilized herein describes the ability of a particular biomolecular "target" (i.e., a biochemical species intended to be inhibited by a particular inhibitor) to perform its biochemical function in the presence of the inhibitor. Biochemical vitality is a function of at least two variables: the ability of a particular inhibitor to inhibit a biochemical target of the replicating biological entity in question, and the ability of the cell's biochemical target to inherently perform its biochemical function (irrespective of an inhibitor). Biochemical vitality also can include other factors that effect the ability of a biochemical target to perform its biochemical function in the presence of the inhibitor.

The biochemical target in question can include, for example, a biochemical species with one or more known or unknown biological functions. The biochemical target can be, for example, a biochemical species having one or more specific biochemical function, or it can be a biochemical species that effects or influences a biochemical function

directly or indirectly. Suitable biochemical targets
include, for example, enzymes, proteins, oligomers,
receptors, and the like. Suitable enzymes include, for
example, reverse transcriptases, proteases (e.g.,
5   retroviral proteases, plasmepsins, and the like),
methylases, oxidases, esterases, acyl transferases, and
the like. Suitable enzymes also include, for example,
viral and non-viral helicases, topoisomerases, DNA
gyrases, DNA and RNA polymerases, parasite-encoded
10 proteases, and the like.

Suitable proteins include, for example, proteins
that incorporate a conformational change as a major
functional requirement, and the like. Examples of such
proteins include HIV gp41 and other fusogenic viral
15 proteins and peptides, topoisomerases, and all DNA
enzymes, and the like.

Suitable oligomers include, for example, oligomers
that require oligomerization in order to perform their
biochemical function. Examples of such oligomers include
20 HIV protease, retroviral fusion proteins, peptides, HIV
gp 41, viral and non-viral membrane fusion proteins,
tumor suppressor proteins (e.g., p53, and the like)
prions, ribosomes, and the like.

The ability of a particular inhibitor to inhibit a
25 biochemical target of a particular replicating biological
entity can be determined by any suitable method and/or
can be obtained from any suitable source. The ability of
a particular inhibitor to inhibit a biochemical function
of a replicating biological entity can be determined, for
30 example, on the basis of a measurable property, or a
measurable relationship of properties, that correlate

with the ability of the inhibitor to inhibit the target.
Suitable methods for determining the ability of the
inhibitor to inhibit the target include, for example,
assays, and the like. In some instances, the ability of

5   the inhibitor to inhibit the target can be obtained from
one or more suitable sources, for example, assay data
from a database, a textbook, or the literature.

When the biochemical target is a protein, the
ability of an inhibitor to inhibit the protein can be

10 determined, for example, by obtaining the equilibrium
dissociation constant (K_d) of drug binding to the target
where drug binding interferes with the function of the
protein.

When the biochemical target is an enzyme, the

15 ability of an inhibitor to inhibit the enzyme can be
determined, for example, by obtaining the inhibition
constant (K_{inh}), or the like. The inhibition constant can
be in terms of drug inhibition constant for the effect of
the drug on substrate catalysis (e.g., K_i) or

20 dissociation constant for drug binding (e.g., K_d) where
drug binding correlates with inhibition of enzyme
function.

When the biochemical target is an oligomer, the
ability of an inhibitor to inhibit the oligomer can be

25 determined, for example, by obtaining the equilibrium
dissociation constant (K_d) for drug binding where drug
binding interferes with oligomerization of the target.

Where the biochemical target is a protein that
requires a conformational change for its function, the

30 ability of an inhibitor to inhibit the conformational
change can be determined, for example, by obtaining the

equilibrium dissociation constant (K_d) for drug binding where drug binding interferes with the conformational change of the target.

When the biochemical target is a protein that is
5   required to bind to a ligand, macromolecule, or macromolecular complex to perform its biochemical function, the ability of an inhibitor to inhibit the protein function can be determined by obtaining the equilibrium dissociation constant (K_d) for drug binding
10   where drug binding interferes with ligand binding, macromolecule binding, or macromolecular complex binding.

When the biochemical target is a nucleic acid binding protein, the ability of an inhibitor to inhibit the nucleic acid binding protein's function can be
15   determined by obtaining the equilibrium dissociation constant (K_d) for drug binding where drug binding interferes with nucleic acid binding.

Vitality also is a function of the biochemical target's ability to inherently perform its biochemical
20   function (irrespective of an inhibitor). The biochemical target's ability to inherently perform its biochemical function can be determined by any suitable method and/or can be obtained from any suitable source. The biochemical target's ability to inherently perform its
25   biochemical function can be determined, for example, on the basis of a measurable property, or measurable relationship of properties, that correlate with the ability of the biochemical target's ability to inherently perform its biochemical function. Suitable methods for
30   determining the biochemical target's ability to inherently perform its biochemical function include, for

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example, biochemical assays, and the like. In some
instances, the ability of a cell's biochemical target to
inherently perform its biochemical function can be
obtained from one or more suitable sources, for example,

5   assay data from a database, a textbook, or the
literature.

When the biochemical target is an enzyme, the
ability of the enzyme to inherently perform its
biochemical function can be determined, for example, by

10   determining the catalytic efficiency of the enzyme. For
example, the catalytic efficiency for enzymes that
exhibit Michaelis-Menten kinetics can be determined by
obtaining the k_{cat}/K_M ratio, or by a similar method,
wherein k_{cat} is the catalytic rate and K_M is the Michaelis

15   constant.

When the biochemical target is a protein, the
ability of the protein to inherently perform its
biochemical function can be determined, for example, by
obtaining the equilibrium constant (K_{eq}) for the

20   biochemical function of the protein, or the like.

When the biochemical target is an oligomer, the
ability of an inhibitor to perform its biological
function can be determined, for example, by obtaining the
equilibrium constant (K_{eq}) that is associated with

25   oligomerization.

Where the biochemical target is a protein that
requires a conformational change for its function, the
ability of the target to perform its function can be
determined, for example, by obtaining the equilibrium

30   constant (K_{eq}) associated with conformational change.

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When the biochemical target is a protein that is
required to bind to a ligand to perform its function, the
ability of the target to perform its function can be
determined, for example, by obtaining the equilibrium
5   dissociation constant (K_d) for ligand binding.

When the biochemical target is a nucleic acid
binding protein, the ability of an inhibitor to perform
its function can be determined by obtaining the
equilibrium dissociation constant (K_d) for nucleic acid
10   binding.

It will be appreciated that vitality also can be a
function of other factors that effect the ability of a
biochemical target to perform its biochemical function in
the presence of the inhibitor. If the biochemical target
15   is a dimeric species, for example, other factors that
influence biochemical vitality might include the ability
of the species to dimerize in the presence and/or in the
absence of the inhibitor. If, by way of example, a
mutation causes the dimerization rate to become a factor
20   in the biochemical function of the biochemical target of
the mutant relative to its predecessor's, then
dimerization rate can be included in the vitality
determination.

The biochemical vitalities of a mutant replicating
25   biological entity and its predecessor, when compared,
describes the biochemical fitness of the target of the
mutant cell. In keeping with the invention, it has been
found that the biochemical fitness relates to the
biological fitness of the mutant in the presence of the
30   inhibitor. When the value for the biochemical vitality
of the target of the mutant exceeds the value for the

biochemical vitality of the target of a predecessor of
the mutant, the target of the mutant has greater
biochemical fitness in the presence of the inhibitor. In
such cases, the mutant replicating biological entity is

5   favored over the predecessor and resistance to the
inhibitor that is used to treat the predecessor is likely
to develop.

Biochemical vitality can be determined in many
different ways that suitably relate the various factors

10   relating to the biochemical vitality of the target. For
example, a mathematical function may be used to relate
the various factors. By way of illustration, when the
biochemical target is an enzyme, the vitality can be
determined as a function of K_{inh} (e.g., K_i or K_d) and

15   enzymatic or catalytic efficiency (e.g., K_{cat}/K_M).
Vitality can be determined as the product of K_{inh} and
enzymatic efficiency, for example, (K_{inh}) x (catalytic
efficiency), or (K_i) x (catalytic efficiency) or
(K_d)(catalytic efficiency). Alternatively, vitality can

20   be determined, for example, as the log of the product of
K_{inh} and enzymatic efficiency, for example, $\log[(K_{inh}) \times$
(catalytic efficiency)], or $\log[(K_i) \times$ (catalytic
efficiency)] or $\log[(K_d) \times$ (catalytic efficiency)].
Similarly, for enzymes that exhibit Michaelis-Menten

25   kinetics, vitality can be determined as a function of K_{inh}
(e.g., K_i or K_d) and the k_{cat}/K_M ratio. For example,
vitality can be determined as the product of K_{inh} and
k_{cat}/K_M, e.g., (K_{inh}) x (k_{cat}/K_M), wherein K_{inh} is K_i or K_d.
Alternatively, vitality can be determined, for example,

30   as the log of the product of K_{inh} and k_{cat}/K_M, e.g.,
$\log[(K_{inh}) \times (k_{cat}/K_M)]$, wherein K_{inh} is K_i or K_d. In a

preferred embodiment, the biochemical target is an enzyme
and the vitality is (K_i) x (k_{cat}/K_M), or $\log[(K_i)$ x
$(k_{cat}/K_M)]$.

5   "Fitness," unless otherwise indicated, means
biochemical fitness. "Biochemical fitness" as utilized
herein is a value that represents the vitality of a
biochemical target of a mutant replicating biological
entity relative to the vitality the biochemical target of
its predecessor. Biochemical fitness is determined by
10   comparing the vitality of a biochemical target of a mutant
replicating biological entity relative to that of its
predecessor. Any suitable comparison of the vitality of a
biochemical target of a mutant replicating biological
entity relative to that of its predecessor can be used in
15   the determination of fitness. For example, biochemical
fitness can be determined as the difference between the
biochemical vitality of a biochemical target of a
predecessor (biochemical vitality$_{pred}$) and the biochemical
vitality of the biochemical target of a particular mutant
20   replicating biological entity that can evolve from the
predecessor (biochemical vitality$_{mut}$), e.g., (biochemical
vitality$_{mut}$) - (biochemical vitality$_{pred}$). If biochemical
fitness is determined on the basis of this difference,
then a positive value indicates that the mutant has a
25   higher fitness relative to its predecessor in the presence
of the inhibitor, whereas a negative value indicates that
the mutant is less fit relative to its predecessor. A
value of zero indicates that the fitness of the mutant and
the predecessor are equal. A higher positive value
30   indicates a greater chance that resistance to the
inhibitor will emerge, whereas a higher negative value

indicates a lower chance that resistance to the inhibitor
will emerge.

Alternatively, and preferably, fitness can be
determined as the quotient of two biochemical vitalities,
for example, as the quotient of a biochemical target of a
particular mutant replicating biological entity and the
biochemical vitality of the biochemical target of a
predecessor, e.g.,

$$\text{fitness} = \frac{\text{vitality}_{\text{mut}}}{\text{vitality}_{\text{pred}}}.$$

If fitness is determined on the basis of this quotient,
then a value greater than one indicates that the mutant
has a higher fitness relative to its predecessor, in the
presence of the inhibitor. A value of one indicates that
the fitness of the mutant and the predecessor are equal.
A value less than one indicates that the mutant is less
fit relative to its predecessor. A higher value indicates
a greater chance that resistance to the inhibitor/drug
will emerge, whereas a lower value indicates a lower
chance that resistance to the inhibitor/drug will emerge.
A value less than one indicates that the mutant will not
emerge in the presence of the inhibitor/drug.

Alternatively, fitness can be determined as the log
of the quotient of two biochemical vitalities, for
example, as the log of the quotient of a biochemical
target of a particular mutant replicating biological
entity and the biochemical vitality of the biochemical
target of a predecessor, e.g.,

$$\text{fitness} = \log\left[\frac{\text{vitality}_{\text{mut}}}{\text{vitality}_{\text{pred}}}\right].$$

If fitness is determined on the basis of this log, then a value greater than zero indicates that the mutant has a higher fitness relative to its predecessor, in the presence of the inhibitor. A negative value indicates that the mutant is less fit relative to its predecessor. A value of zero indicates that the fitness of the mutant and the predecessor are equal. A higher positive value indicates a greater chance that resistance to the inhibitor/drug will emerge, whereas a lower positive value indicates a lower chance that resistance to the inhibitor/drug will emerge. A negative value indicates that the mutant will not emerge in the presence of the inhibitor/drug.

Fitness can be determined in the presence of any suitable compound that inhibits a biochemical target from performing its biological function. The inhibitor, for example, can be a compound that inhibits an enzyme. Suitable enzyme inhibitors include, for example, protease inhibitors, reverse transcriptase inhibitors, DNA polymerase inhibitors, methylase inhibitors, oxidase inhibitors, esterase inhibitors, acyl transferase inhibitors, and the like.

Suitable protease inhibitors include, for example, viral protease inhibitors, plasmepsin inhibitors, and cathepsin D inhibitors. In a preferred embodiment, the inhibitor is a viral protease inhibitor, more preferably a retroviral protease inhibitor, still more preferably an HIV-1 or an HIV-2 protease inhibitor, and most preferably and HIV-1 protease inhibitor. Exemplary HIV-1 protease inhibitors include, for example, saquinavir, ritonavir, indinavir, nelfinavir, amprenavir, and HIV-1 protease

inhibitors that are undergoing clinical trials, e.g.,
tipranavir (PNU-140690).

Suitable plasmepsin inhibitors include, for example,
inhibitors of plasmepsin I or II, including inhibitors of
plasmepsin I or II that have antimalarial activity.
Suitable inhibitors of cathepsin D include, for example,
cathepsin D inhibitors that inhibit cathepsin D in
primary breast cancer tissues, including cathepsin D
inhibitors that inhibit cathepsin D in primary breast
cancer tissues and would be expected to lower the risk of
metastasis and/or shorter relapse-free survival in breast
cancer patients. *See, e.g.*, Gulnik et al., *J. Mol.
Biol.*, *227*, 265-270 (1992).

Suitable reverse transcriptase inhibitors include,
for example, retroviral reverse transcriptase inhibitors,
e.g., AZT, 3TC, ddI, ddC, D4T, and the like.

Suitable protein inhibitors include, for example,
compounds that inhibit a conformational change in a
protein, and the like. Suitable oligomerization
inhibitors include, for example, T-20 peptide inhibitor
of HIV-1 fusion and other compounds that inhibit
oligomers from oligomerizing on a cell surface or within
a cell membrane.

In accordance with the present invention, fitness in
the presence of an inhibitor can be determined for a
biological entity that produces or includes a biological
target of the inhibitor. The biological entity is
preferably a replicating biological entity, for example, a
virus, a parasite, or a cell, preferably a disease-causing
cell. Disease-causing replicating biological entities
include, for example, tumor cells, cancer cells, and

infectious organisms (e.g., fungi, protozoa, bacteria, and the like) and prions.

Cancer cells include, for example, cells associated with breast cancer, colon cancer, lung cancer, and the like. Fitness can be determined for a rapidly growing tumor cell.

Fungi include, for example, candida albicans, and the like. Protozoa include, for example, trypanosome species, schistosomial species, malarial protozoa, e.g., Plasmodium species. Plasmodium species include, for example, Plasmodium Falciparum, Plasmodium ovale, Plasmodium vivax, Plasmodium malariae, and the like. Bacteria include, for example, Helicobacter pylori, Escherichia coli, Salmonella, Streptococcus pyogenes, Staphylococcus aureas, Bacillus anthrax, Mycobacterium tuberculosis, Hemophilus influenza, and the like. Viruses include, for example, retroviruses (e.g., HIV-1 and HIV-2), herpes viruses, cytomegaloviruses, influenza viruses, epstein-barr virus (EBV), Kaposi's sarcoma herpes virus (KSHV), varicella-zoster virus (VZV), human papillomavirus (HPV), echovirus, picornaviruses, rhinoviruses, poliovirus, coxsackie virus, measles, mumps, human T-cell leukemia virus (HTLV-1), rubella, rotaviruses, yellow fever virus, ebola virus, and other pathogenic viruses, and the like.

Replicating biological entities also include multicellular organisms, for example, infectious microorganisms, e.g., helminths. Helminths include, for example, hookworms (e.g., ancylostoma duodenale) strongyloides stercoralis, fasciola hepatica, trichuris trichiura, trichinella spiralis, taenia solium, taenia saginata, and the like.

It is believed that drug resistance is the
evolutionary result of fitness-based selection of mutant
cells/microorganisms in the presence of a drug (or any
compound that has biological activity). In accordance

5   with the present invention, the emergence (or non-
emergence) of drug resistance in a disease caused by a
disease-causing replicating biological entity can be
predicted by determining the fitness of a biochemical
target of a mutant in the presence of the drug. Thus, the

10 emergence (or non-emergence) of drug resistance can be
predicted on the basis of biochemical fitness. While
resistance profiles may, in some instances, reflect
fitness, it cannot be assumed that the emergence of drug
resistance for a particular mutant can be directly

15 predicted on the basis of its resistance profile alone.

    The present invention thus provides an assay that can
be used to predict the biological fitness of a replicating
biological entity in the presence of a particular
inhibitor. In a preferred embodiment, an assay is

20 provided for determining the biochemical fitness of a
biochemical target of a mutant replicating biological
entity relative to its predecessor. In accordance with
the assay of the present invention, a predecessor to the
mutant is obtained, the biochemical vitality of the

25 biochemical target of the predecessor in the presence of
a compound capable of inhibiting the biochemical target
of the predecessor is determined, the biochemical
vitality of the biochemical target of the mutant
in the presence of the compound is determined, and the

30 biochemical vitality of the biochemical target of the

mutant relative to the biochemical vitality of the
biochemical target of the predecessor are compared.

The assay can be used with a wide variety of
infectious microorganisms, as described above, including,
for example, a virus, a fungus, a protozoa, or bacterium,
a retrovirus, including HIV-1 or HIV-2, and cancer cells.
When the infectious microorganism is a protozoa, it is
preferably a malarial parasite, which is more preferably
a plasmodium species.

In another embodiment, the predecessor is a cancer
cell, which is preferably a rapidly growing tumor cell,
for example, a rapidly growing cancer cell found in
breast cancer, colon cancer, lung cancer, a tumor cell of
a lymphoid origin, a tumor-derived cell with a high
metastatic potential, or the like.

The assay of the present invention can be applied to
any suitable biochemical target, preferably a biochemical
target whose biochemical vitality can be determined using
measurable properties that can be obtained by assay.
Desirably, the biochemical target is one that plays an
important role in the replication and growth of the
entity. By way of example, the biochemical target of the
predecessor (and the mutant) can be an enzyme and the
compound can be an inhibitor of the enzyme of the
predecessor.

The enzyme can be a viral enzyme. Illustrative of
viral enzymes are a viral protease enzyme, a viral
reverse transcriptase, a viral integrase, a viral
polymerase, a viral protein with enzymatic activity, or a
retroviral enzyme, including an HIV-1 or an HIV-2 enzyme.
Viral protease enzymes, include a retroviral protease,

such as an HIV-1 protease or an HIV-2 protease. Viral
integrase enzymes include, for example, HIV-1 integrase,
HIV-2 integrase, and the like. Viral polymerase can be a
retroviral polymerase, including an HIV-1 polymerase or

5   an HIV-2 polymerase. A viral protein with enzymatic
activity can be a retroviral protein, such as an HIV-1
protein or an HIV-2 protein.

The enzyme also can be a protozoal enzyme, including
a protozoal protease enzyme. The protozoal protease can

10   be a malarial protease. The malarial protease can be a
plasmepsin, including plasmepsin I or plasmepsin II. The
malarial enzyme can also be a plasmodial enzyme or a
protein with enzymatic activity.

In yet another embodiment, the biochemical target of

15   the predecessor is an oligomer and the compound inhibits
the oligomerization of the oligomer of the predecessor.
In yet another embodiment, the biochemical target of the
predecessor is a protein and the compound inhibits a
conformational change in the protein of the predecessor.

20   The biochemical vitality determination can also take
into account other factors, preferably measurable
factors, that effect the ability of a biochemical target
to perform its biochemical function in the presence of
the inhibitor. When the biochemical target is an enzyme

25   and the compound is an enzyme inhibitor, the biochemical
vitality of the enzyme of the mutant replicating
biological entity preferably corresponds to $K_{inh-mut}$, $k_{cat-mut}$,
K_{M-mut}, and the biochemical vitality of the enzyme of the
predecessor preferably corresponds to $K_{inh-pred}$, $k_{cat-pred}$, and

30   K_{M-pred}. K_{inh} is an inhibition constant of the compound, k_{cat}
is the biochemical catalytic rate, and K_M is the

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Michaelis constant. More preferably, the vitality of the
enzyme corresponds to K_{inh}, k_{cat} and K_M, and the biochemical
vitality of the enzyme of the mutant replicating
biological entity is defined by the relationship $K_{inh\text{-}mut}(k_{cat\text{-}mut}/K_{M\text{-}mut})$ (i.e., $(K_{inh\text{-}mut}) \times (K_{cat\text{-}mut}/K_{M\text{-}mut}))$ and the
biochemical vitality of the enzyme of the predecessor is
defined by the relationship $K_{inh\text{-}pred}(k_{cat\text{-}pred}/K_{M\text{-}pred})$. The
variables $K_{inh\text{-}mut}$, $K_{inh\text{-}pred}$, $k_{cat\text{-}mut}$, $k_{cat\text{-}pred}$, $K_{M\text{-}mut}$, and $K_{M\text{-}pred}$
can be obtained by any suitable means, and are preferably
obtained by measurement (e.g., from an assay). When
vitality is determined on the basis of these
relationships, biochemical fitness in the presence of a
given inhibitor/drug preferably is defined by the
equation:

$$\frac{K_{inh\text{-}mut}(k_{cat\text{-}mut}/K_{M\text{-}mut})}{K_{inh\text{-}pred}(k_{cat\text{-}pred}/K_{M\text{-}pred})}, \text{ or}$$

$$\log\left[\frac{K_{inh\text{-}mut}(k_{cat\text{-}mut}/K_{M\text{-}mut})}{K_{inh\text{-}pred}(k_{cat\text{-}pred}/K_{M\text{-}pred})}\right].$$

K_{inh} can be determined by any suitable means, but
typically is determined on the basis of K_i or K_d.

The present invention also provides a method of
administering a therapeutic compound, which method
increases the chances of successful long-term therapy.
In a preferred embodiment, the present invention provides
a method of administering a therapeutic compound that
inhibits a biochemical target of a replicating disease-
causing replicating biological entity (disease causing
predecessor), including identifying at least one mutant
capable of evolving from the disease-causing predecessor.

A first biochemical vitality of the biochemical target of the disease-causing predecessor in the presence of a first compound capable of inhibiting the biochemical target of the disease-causing predecessor, and a first

5   biochemical vitality of the biochemical target of the mutant in the presence of the first compound, are determined.

Additional biochemical vitalities of the biochemical target of the disease-causing replicating biological

10   entity in the presence of additional compounds capable of inhibiting the biochemical target of the disease-causing cell, and additional biochemical vitalities of the biochemical target of the mutant in the presence of the additional compounds, are also determined.

15   Fitnesses in the presence of different inhibitors/drugs can be compared and a therapeutic compound administered on the basis of the comparison. A first biochemical fitness of the biochemical target of the mutant relative to the disease-causing predecessor is

20   determined by comparing the first biochemical vitality of the biochemical target of the mutant with the first biochemical vitality of the biochemical target of the disease-causing predecessor, and a second biochemical fitness of the biochemical target of the mutant relative

25   to the disease-causing replicating biological entity is determined by comparing the second biochemical vitality of the biochemical target of the mutant with the second biochemical vitality of the biochemical target of the disease-causing replicating biological entity.

30   Additional biochemical fitness determinations can be made in the presence of additional compounds. The biochemical

fitness values for one or more mutants in the presence of
each compound are compared. A therapeutic compound is
then administered from among the first and the additional
compound(s), which therapeutic compound produces the

5   lowest biochemical fitness values.

In accordance with the method of the present
invention, the replicating disease-causing replicating
biological entity is less likely to develop resistance in
the presence of the therapeutic compound. The

10 therapeutic compound can be administered from among any
particular set of compounds, which can have the same
biochemical target or different biochemical targets with
respect to each other. The method of administering a
compound in accordance with the present invention is,

15 therefore, not limited to comparing fitness in the
presence of compounds that act on the same biochemical
target.

In one embodiment, the disease-causing replicating
biological entity is an infectious microorganism, for

20 example, a virus, a fungus, a protozoa, or a bacterium,
more preferably a virus or a protozoa. When the
infectious microorganism is a virus, it is preferably a
retrovirus, which is more preferably HIV-1 or HIV-2, and
most preferably HIV-1. When the infectious microorganism

25 is a protozoa, it is preferably a malarial parasite,
which is more preferably a plasmodium species.

In another embodiment, the disease-causing
replicating biological entity is a cancer cell, which is
preferably a rapidly growing tumor cell, for example, a

30 rapidly growing cancer cell found in breast cancer, colon
cancer, lung cancer, or the like.

The method of administering a compound in accordance
with the present invention can be applied to any suitable
biochemical target, preferably a biochemical target whose
biochemical vitality can be determined using measurable
5   properties that can be obtained by assay. In one
embodiment, the biochemical target of the predecessor
(and the mutant) is an enzyme and the compound inhibits
an enzyme of the predecessor. The enzyme can be any
enzyme whose biochemical vitality can be measured
10   including, for example, an enzyme described herein in
connection with the fitness assay of the present
invention.

In another embodiment, the biochemical target of the
disease-causing replicating biological entity is an
15   oligomer and the compound inhibits the oligomerization of
the oligomer of the predecessor. In yet another
embodiment, the biochemical target of the disease-causing
replicating biological entity is a protein and the
compound inhibits a conformational change in the protein
20   of the predecessor.

The biochemical vitality can be determined in any
suitable manner. For example, vitality can be determined
as described herein, e.g., as described in connection
with the assay of the present invention.

25   When an infectious microorganism is tested in
accordance with the assay of the present invention, the
predecessor can be a wild-type species, or the
predecessor can itself be a mutant species. In a
particularly preferred embodiment, the predecessor is a
30   retrovirus, which is more preferably a wild-type HIV-1 or
HIV-2 strain, most preferably HIV-1. When the

predecessor is a wild-type HIV strain, the mutant
replicating biological entity preferably has at least one
mutation in the biochemical target thereof. When the
predecessor has at least one mutation in the biochemical

5   target thereof, the mutant preferably has at least two
mutations in the biochemical target thereof.

Similarly, when the method of administering a
therapeutic compound in accordance with the present
invention is used in connection with an infectious

10   microorganism, the disease-causing replicating biological
entity can be a wild-type species, or the disease-causing
entity can itself be a mutant species. In a particularly
preferred embodiment, the disease-causing replicating
biological entity is a retrovirus, which is more

15   preferably a wild-type HIV-1 or HIV-2 strain, most
preferably HIV-1. When the disease-causing replicating
biological entity is a wild-type HIV strain, the mutant
preferably has at least one mutation in the biochemical
target thereof. When the disease-causing replicating

20   biological entity has at least one mutation in the
biochemical target thereof, the mutant preferably has at
least two mutations in the biochemical target thereof.

When the predecessor or the disease-causing
replicating biological entity in the assay of the present

25   invention, or in the method of administering a compound
in accordance with the present invention, is a wild-type
HIV strain, the biochemical target of the mutant
preferably has at least one active site mutation. When
the predecessor in the assay of the present invention has

30   at least one mutation, and the mutant replicating
biological entity has at least two mutations, the

biochemical target of the predecessor or of the mutant

preferably has at least one active site mutation. When

the disease-causing replicating biological entity in the

method of the present invention has at least one mutation

5   in the biochemical target thereof, and the mutant has at

least two mutations in the biochemical target thereof,

the biochemical target of the disease-causing entity or

of the mutant preferably has at least one active site

mutation.

10   The present invention further provides a continuous

fluorogenic assay for measuring the anti-HIV protease

activity of a protease inhibitor, which method comprises

adding a solution of HIV protease to a substrate stock

solution, in which the substrate has the formula Ala-Arg-

15   Val-Tyr-Phe(NO_2)-Glu-Ala-Nle-NH_2, to provide a substrate

reaction solution. The fluorescence of the substrate

reaction solution is then measured at specified time

intervals. The solution of HIV protease is then added to

a solution of the protease inhibitor and the substrate

20   stock solution, to provide an inhibitor-substrate

reaction solution. The fluorescence of the inhibitor-

substrate reaction solution is then measured at specified

time intervals. The initial velocity of the inhibitor-

substrate reaction solution is then calculated by

25   applying the equation:

$V = V_0/2E_t (\{ [K_i(1+S/K_m)+I_t-E_t]^2 + 4K_i(1+S/K_m)E_t\}^{1/2} - [K_i((1+S/K_m)+I_t-E_t])$, wherein V is the initial velocity of the inhibitor

reaction solution, V_0 is the initial velocity of the

substrate reaction solution, K_m is the Michaelis-Menten

30   constant, S is the substrate concentration, E_t is the

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protease concentration, and I_t is the inhibitor

concentration.

The assay method described herein is highly

sensitive and particularly useful for the prediction of

5   the antiviral inhibitory activity of a compound against

mutant HIV, more particularly multiple mutant HIV,

specifically multidrug-resistant human immunodeficiency

viruses. The continuous flourogenic assay of the present

invention is distinctly advantageous in that it is more

10   sensitive than standard assays in determining the

activity of protease inhibitors against multidrug-

resistant HIV. The continuous flourogenic assay of the

present invention is disclosed in more detail in the

examples that follow. The inhibitory data obtained in

15   accordance with this continuous fluorogenic assay can be

used to determine vitality and fitness for HIV-1 protease

in the presence of a protease inhibitor, in accordance

with the present invention.

The present invention also provides a method of

20   preventing the emergence of drug resistance in an HIV-

infected mammal that includes the administration of a drug

resistance-inhibiting effective amount of a compound

represented by the formula:
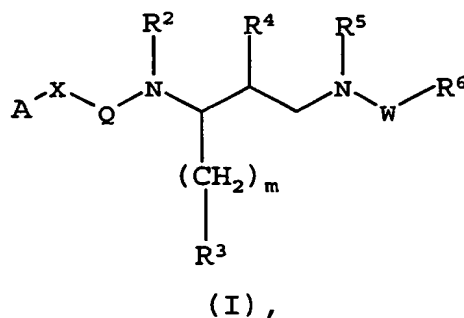
$$A^{-X}\diagdown Q^{-N}\diagup \underset{\underset{\displaystyle R^3}{\overset{\displaystyle |}{\underset{(CH_2)_m}{|}}}{\overset{R^2}{|}} \diagdown \overset{R^4}{\underset{|}{}} \diagup \overset{R^5}{\underset{|}{N}} \diagdown_W{}^{-R^6}$$

25   (I),

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or a pharmaceutically acceptable salt, a prodrug, or an
ester thereof, or a pharmaceutical composition thereof,
wherein:

A is a group of the formula:

5
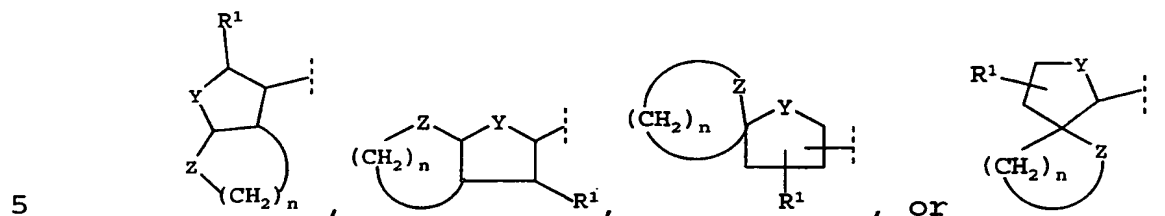
R^1 is H or an alkyl, an alkenyl, an alkynyl, a
cycloalkyl, a cycloalkylalkyl, an aryl, an aralkyl, a
heterocycloalkyl, a heterocycloalkylalkyl, a heteroaryl,
or a heteroaralkyl radical, in which at least one

10   hydrogen atom is optionally substituted with a
substituent independently selected from the group
consisting of OR^7, SR^7, CN, NO_2, N_3, and a halogen, wherein
R^7 is H, an alkyl, an alkenyl, or an alkynyl;

Y and Z are the same or different and are

15   independently selected from the group consisting of CH_2,
O, S, SO, SO_2, NR^8, $R^8C(O)N$, $R^8C(S)N$, $R^8OC(O)N$, $R^8OC(S)N$,
$R^8SC(O)N$, $R^8R^9NC(O)N$, and $R^8R^9NC(S)N$, wherein R^8 and R^9 are
independently selected from the group consisting of H, an
alkyl, an alkenyl, and an alkynyl;

20   n is an integer from 1 to 5;

X is a covalent bond, CHR^{10}, $CHR^{10}CH_2$, CH_2CHR^{10}, O,
NR^{10}, or S, wherein R^{10} is H, an alkyl, an alkenyl, or an
alkynyl;

Q is C(O), C(S), or SO_2;

25   R^2 is H, an alkyl, an alkenyl, or an alkynyl;

m is an integer from 0 to 6;

R^3 is a cycloalkyl, a heterocycloalkyl, an aryl, or
a heteroaryl in which at least one hydrogen atom is

optionally substituted with a substituent independently
selected from the group consisting of H, alkyl, $(CH_2)_pR^{11}$,
OR^{12}, SR^{12}, CN, N_3, NO_2, $NR^{12}R^{13}$, $C(O)R^{12}$, $C(S)R^{12}$, CO_2R^{12},
$C(O)SR^{12}$, $C(O)NR^{12}R^{13}$, $C(S)NR^{12}R^{13}$, $NR^{12}C(O)R^{13}$, $NR^{12}C(S)R^{13}$,
5    $NR^{12}CO_2R^{13}$, $NR^{12}C(O)SR^{13}$, and a halogen, wherein:

p is an integer from 0 to 5;

R^{11} is a cycloalkyl, a heterocycloalkyl, an
aryl, or a heteroaryl in which at least one hydrogen atom
is optionally substituted with a substituent
10   independently selected from the group consisting of a
halogen, OH, OCH_3, NH_2, NO_2, SH, and CN; and

R^{12} and R^{13} are independently selected from the
group consisting of H, an alkyl, an alkenyl, and an
alkynyl;

15   R^4 is OH, =O (keto), or NH_2, wherein, when R^4 is OH,
it is optionally in the form of a pharmaceutically
acceptable ester or prodrug, and when R^4 is NH_2, it is
optionally an amide, a hydroxylamino, a carbamate, a
urea, an alkylamino, a dialkylamino, a protic salt, or a
20   tetraalkylammonium salt;

R^5 is H, a C_1-C_6 alkyl radical, a C_2-C_6 alkenyl
radical, or $(CH_2)_qR^{14}$, wherein q is an integer form 0 to 5,
and R^{14} is a cycloalkyl, a heterocycloalkyl, an aryl, or a
heteroaryl radical in which at least one hydrogen atom is
25   optionally substituted with a substituent independently
selected from the group consisting of a halogen, OH,
OCH_3, NH_2, NO_2, SH, and CN;

W is C(O), C(S), S(O), or SO_2; and

R^6 is a cycloalkyl, heterocycloalkyl, aryl, or
30   heteroaryl radical in which at least one hydrogen atom is
optionally substituted with a substituent independently

selected from the group consisting of a halogen, OR^{15},
SR^{15}, $S(O)R^{15}$, SO_2R^{15}, $SO_2NR^{15}R^{16}$, $SO_2N(OH)R^{15}$, CN, $CR^{15}=NR^{16}$,
$CR^{15}=N(OR^{16})$, N_3, NO_2, $NR^{15}R^{16}$, $N(OH)R^{15}$, $C(O)R^{15}$, $C(S)R^{15}$,
CO_2R^{15}, $C(O)SR^{15}$, $C(O)NR^{15}R^{16}$, $C(S)NR^{15}R^{16}$, $C(O)N(OH)R^{15}$,

5    $C(S)N(OH)R^{15}$, $NR^{15}C(O)R^{16}$, $NR^{15}C(S)R^{16}$, $N(OH)C(O)R^{15}$,
$N(OH)C(S)R^{15}$, $NR^{15}CO_2R^{16}$, $N(OH)CO_2R^{15}$, $NR^{15}C(O)SR^{16}$,
$NR^{15}C(O)NR^{16}R^{17}$, $NR^{15}C(S)NR^{16}R^{17}$, $N(OH)C(O)NR^{15}R^{16}$,
$N(OH)C(S)NR^{15}R^{16}$, $NR^{15}C(O)N(OH)R^{16}$, $NR^{15}C(S)N(OH)R^{16}$,
$NR^{15}SO_2R^{16}$, $NHSO_2NR^{15}R^{16}$, $NR^{15}SO_2NHR^{16}$, $P(O)(OR^{15})(OR^{16})$, an

10   alkyl, an alkoxy, an alkylthio, an alkylamino, a
cycloalkyl, a cycloalkylalkyl, a heterocycloalkyl, a
heterocycloalkylalkyl, an aryl, an aryloxy, an arylamino,
an arylthio, an aralkyl, an aryloxyalkyl, an
arylaminoalkyl, an aralkoxy, an (aryloxy)alkoxy, an

15   (arylamino)alkoxy, an (arylthio)alkoxy, an aralkylamino,
an (aryloxy)alkylamino, an (arylamino)alkylamino, an
(arylthio)alkylamino, an aralkylthio, an
(aryloxy)alkylthio, an (arylamino)alkylthio, an
(arylthio)alkylthio, a heteroaryl, a heteroaryloxy, a

20   heteroarylamino, a heteroarylthio, a heteroaralkyl, a
heteroaralkoxy, a heteroaralkylamino, and a
heteroaralkylthio,

        wherein R^{15}, R^{16}, and R^{17} are H, an unsubstituted
alkyl, and an unsubstituted alkenyl,

25   wherein, when at least one hydrogen atom of R^6
is optionally substituted with a substituent other than a
halogen, OR^{15}, SR^{15}, $S(O)R^{15}$, SO_2R^{15}, $SO_2NR^{15}R^{16}$, $SO_2N(OH)R^{15}$,
CN, $CR^{15}=NR^{16}$, $CR^{15}=N(OR^{16})$, N_3, NO_2, $NR^{15}R^{16}$, $N(OH)R^{15}$, $C(O)R^{15}$,
$C(S)R^{15}$, CO_2R^{15}, $C(O)SR^{15}$, $C(O)NR^{15}R^{16}$, $C(S)NR^{15}R^{16}$,

30   $C(O)N(OH)R^{15}$, $C(S)N(OH)R^{15}$, $NR^{15}C(O)R^{16}$, $NR^{15}C(S)R^{16}$,
$N(OH)C(O)R^{15}$, $N(OH)C(S)R^{15}$, $NR^{15}CO_2R^{16}$, $N(OH)CO_2R^{15}$,

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$NR^{15}C(O)SR^{16}$, $NR^{15}C(O)NR^{16}R^{17}$, $NR^{15}C(S)NR^{16}R^{17}$, $N(OH)C(O)NR^{15}R^{16}$,

$N(OH)C(S)NR^{15}R^{16}$, $NR^{15}C(O)N(OH)R^{16}$, $NR^{15}C(S)N(OH)R^{16}$,

$NR^{15}SO_2R^{16}$, $NHSO_2NR^{15}R^{16}$, $NR^{15}SO_2NHR^{16}$, or $P(O)(OR^{15})(OR^{16})$, then

at least one hydrogen atom on said substituent is

optionally substituted with a halogen, OR^{15}, SR^{15}, $S(O)R^{15}$,

SO_2R^{15}, $SO_2NR^{15}R^{16}$, $SO_2N(OH)R^{15}$, CN, $CR^{15}=NR^{16}$, $CR^{15}=N(OR^{16})$, N_3,

NO_2, $NR^{15}R^{16}$, $N(OH)R^{15}$, $C(O)R^{15}$, $C(S)R^{15}$, CO_2R^{15}, $C(O)SR^{15}$,

$C(O)NR^{15}R^{16}$, $C(S)NR^{15}R^{16}$, $C(O)N(OH)R^{15}$, $C(S)N(OH)R^{15}$,

$NR^{15}C(O)R^{16}$, $NR^{15}C(S)R^{16}$, $N(OH)C(O)R^{15}$, $N(OH)C(S)R^{15}$,

$NR^{15}CO_2R^{16}$, $N(OH)CO_2R^{15}$, $NR^{15}C(O)SR^{16}$, $NR^{15}C(O)NR^{16}R^{17}$,

$NR^{15}C(S)NR^{16}R^{17}$, $N(OH)C(O)NR^{15}R^{16}$, $N(OH)C(S)NR^{15}R^{16}$,

$NR^{15}C(O)N(OH)R^{16}$, $NR^{15}C(S)N(OH)R^{16}$, $NR^{15}SO_2R^{16}$, $NHSO_2NR^{15}R^{16}$,

$NR^{15}SO_2NHR^{16}$, or $P(O)(OR^{15})(OR^{16})$.

Optionally, R^5 and R^6 are covalently bonded such that

R^5 and R^6, together with the N-W bond of formula (I),

comprise a 12 to 18 membered ring. The 12 to 18 membered

ring can comprise at least one additional heteroatom in

the ring skeleton other than the nitrogen of the N-W bond

(e.g., N, O, or S) within the ring. In the practice of

the method of preventing the emergence of drug resistance

in an HIV-infected mammal, it is preferable that a mutant

virus that is capable of evolving from the infection has

low fitness, relative to the infecting virus, in the

presence of the compound or combination of compounds that

are administered.

As utilized herein, the term "alkyl" means a

straight-chain or branched alkyl radical containing from

about 1 to about 20 carbon atoms chain, preferably from

about 1 to about 10 carbon atoms, more preferably from

about 1 to about 8 carbon atoms, still more preferably

from about 1 to about 6 carbon atoms. Examples of such

substituents include methyl, ethyl, propyl, isopropyl, *n*-butyl, *sec*-butyl, isobutyl, *tert*-butyl, pentyl, isoamyl, hexyl, octyl, dodecanyl, and the like.

The term "alkenyl" means a straight-chain or
5 branched-chain alkenyl radical having one or more double bonds and containing from about 2 to about 20 carbon atoms chain, preferably from about 2 to about 10 carbon atoms, more preferably from about 2 to about 8 carbon atoms, still more preferably from about 2 to about 6 carbon
10 atoms. Examples of such substituents include vinyl, allyl, 1,4-butadienyl, isopropenyl, and the like.

The term "alkynyl" means a straight-chain or branched-chain alkynyl radical having one or more triple bonds and containing from about 2 to about 20 carbon atoms
15 chain, preferably from about 2 to about 10 carbon atoms, more preferably from about 2 to about 8 carbon atoms, still more preferably from about 2 to about 6 carbon atoms. Examples of such radicals include ethynyl, propynyl (propargyl), butynyl, and the like.

20 The term "alkoxy" means an alkyl ether radical, wherein the term "alkyl" is defined as above. Examples of alkoxy radicals include methoxy, ethoxy, *n*-propoxy, isopropoxy, *n*-butoxy, isobutoxy, *sec*-butoxy, *tert*-butoxy, hexanoxy, and the like.

25 The term "alkylthio" means an alkyl thioether radical, wherein the term "alkyl" is defined as above. Examples of alkylthio radicals include methylthio (SCH_3), ethylthio (SCH_2CH_3), *n*-propylthio, isopropylthio, *n*-butylthio, isobutylthio, *sec*-butylthio, *tert*-butylthio, *n*-
30 hexylthio, and the like.

The term "alkylamino" means an alkyl amine radical,
wherein the term "alkyl" is defined as above. Examples of
alkylamino radicals include methylamino ($NHCH_3$),
ethylamino ($NHCH_2CH_3$), n-propylamino, isopropylamino, n-
5   butylamino, isobutylamino, sec-butylamino, tert-
butylamino, n-hexylamino, and the like.

The term "cycloalkyl" means a monocyclic or a
polycyclic alkyl radical defined by one or more alkyl
carbocyclic rings, which can be the same or different when
10   the cycloalkyl is a polycyclic radical having 3 to about
10 carbon atoms in the carbocyclic skeleton in each ring,
preferably about 4 to about 7 carbon atoms, more
preferably 5 to 6 carbons atoms. Examples of monocyclic
cycloalkyl radicals include cyclopropyl, cyclobutyl,
15   cyclopentyl, cyclohexyl, cycloheptyl, cyclodecyl, and the
like. Examples of polycyclic cycloalkyl radicals include
decahydronaphthyl, bicyclo[5.4.0]undecyl, adamantyl, and
the like.

The term "cycloalkylalkyl" means an alkyl radical as
20   defined herein, in which at least one hydrogen atom on the
alkyl radical is replaced by a cycloalkyl radical as
defined herein. Examples of cycloalkylalkyl radicals
include cyclohexylmethyl, 3-cyclopentylbutyl, and the
like.

25   The term "heterocycloalkyl" means a cycloalkyl
radical as defined herein (including polycyclics), wherein
at least one carbon which defines the carbocyclic skeleton
is substituted with a heteroatom such as, for example, O,
N, or S, optionally comprising one or more double bond
30   within the ring, provided the ring is not heteroaryl as
defined herein. The heterocycloalkyl preferably has 3 to

about 10 atoms (members) in the carbocyclic skeleton of
each ring, preferably about 4 to about 7 atoms, more
preferably 5 to 6 atoms. Examples of heterocycloalkyl
radicals include epoxy, aziridyl, oxetanyl,

5   tetrahydrofuranyl, dihydrofuranyl, piperadyl, piperidinyl,
pyperazyl, piperazinyl, pyranyl, morpholinyl, and the
like.

The term "heterocycloalkylalkyl" means an alkyl
radical as defined herein, in which at least one hydrogen

10   atom on the alkyl radical is replace by a heterocycloalkyl
radical as defined herein. Examples of
heterocycloalkylalkyl radicals include 2-morpholinomethyl,
3-(4-morpholino)-propyl, 4-(2-tetrahydrofuranyl)-butyl,
and the like.

15   The term "aryl" refers to an aromatic carbocyclic
radical, as commonly understood in the art, and includes
monocyclic and polycyclic aromatics such as, for example,
phenyl and naphthyl radicals, optionally substituted with
one or more substituents selected from the group

20   consisting of a halogen, an alkyl, alkoxy, amino, cyano,
nitro, and the like.

The term "aryloxy" means aryl as defined herein,
wherein a hydrogen atom is replaced by an oxygen.
Examples of aryloxy radicals include phenoxy, naphthoxy,

25   4-flourophenoxy, and the like.

The term "arylamino" means aryl as defined herein,
wherein a hydrogen atom is replaced by an amine. Examples
of arylamino radicals include phenylamino, naphthylamino,
3-nitrophenylamino, 4-aminophenylamino, and the like.

30   The term "arylthio" means aryl as defined herein,
wherein a hydrogen atom is replaced by a sulfur atom.

Examples of arylthio radicals include phenylthio,
naphthylthio, 3-nitrophenylthio, 4-thiophenylthio, and the
like.

The term "aralkyl" means alkyl as defined herein,
wherein an alkyl hydrogen atom is replaced by an aryl as
defined herein. Examples of aralkyl radicals include
benzyl, phenethyl, 3-(2-naphthyl)-butyl, and the like.

The term "aryloxyalkyl" means alkyl as defined
herein, wherein an alkyl hydrogen atom is replaced by an
aryloxy as defined herein. Examples of aryloxyalkyl
radicals include phenoxyethyl, 4-(3-aminophenoxy)-1-butyl,
and the like.

The term "arylaminoalkyl" means alkyl as defined
herein, wherein an alkyl hydrogen atom is replaced by an
arylamino as defined herein. Examples of arylaminoalkyl
radicals include phenylaminoethyl, 4-(3-
methoxyphenylamino)-1-butyl, and the like.

The term "aralkoxy" means alkoxy as defined herein,
wherein an alkyl hydrogen atom is replaced by an aryl as
defined herein. Examples of aralkoxy radicals include 2-
phenylethoxy, 2-phenyl-1-propoxy, and the like.

The term "(aryloxy)alkoxy" means alkoxy as defined
herein, wherein an alkyl hydrogen atom is replaced by an
aryloxy as defined herein. Examples of (aryloxy)alkoxy
radicals include 2-phenoxyethoxy, 4-(3-aminophenoxy)-1-
butoxy, and the like.

The term "(arylamino)alkoxy" means alkoxy as defined
herein, wherein an alkyl hydrogen atom is replaced by an
arylamino as defined herein. Examples of
(arylamino)alkoxy radicals include 2-(phenylamino)-ethoxy,
2-(2-naphthylamino)-1-butoxy, and the like.

The term "(arylthio)alkoxy" means alkoxy as defined herein, wherein an alkyl hydrogen atom is replaced by an arylthio as defined herein. Examples of (arylthio)alkoxy radicals include 2-(phenylthio)-ethoxy, and the like.

5   The term "aralkylamino" means alkylamino as defined herein, wherein an alkyl hydrogen atom is replaced by an aryl as defined herein. Examples of aralkylamino radicals include 2-phenethylamino, 4-phenyl-n-butylamino, and the like.

10   The term "(aryloxy)alkylamino" means alkylamino as defined herein, wherein an alkyl hydrogen atom is replaced by an aryloxy as defined herein. Examples of (aryloxy)alkylamino radicals include 3-phenoxy-n-propylamino, 4-phenoxybutylamino, and the like.

15   The term "(arylamino)alkylamino" means alkylamino as defined herein, wherein an alkyl hydrogen atom is replaced by an arylamino as defined herein. Examples of (arylamino)alkylamino radicals include 3-(naphthylamino)-1-propylamino, 4-(phenylamino)-1-butylamino, and the like.

20   The term "(arylthio)alkylamino" means alkylamino as defined herein, wherein an alkyl hydrogen atom is replaced by an arylthio as defined herein. Examples of (arylthio)alkylamino radicals include 2-(phenylthio)-ethylamino, and the like.

25   The term "aralkylthio" means alkylthio as defined herein, wherein an alkyl hydrogen atom is replaced by an aryl as defined herein. Examples of aralkylthio radicals include 3-phenyl-2-propylthio, 2-(2-naphthyl)-ethylthio, and the like.

30   The term "(aryloxy)alkylthio" means alkylthio as defined herein, wherein an alkyl hydrogen atom is replaced

by an aryloxy as defined herein. Examples of
(aryloxy)alkylthio radicals include 3-phenoxypropylthio,
4-(2-fluorophenoxy)-butylthio, and the like.

The term "(arylamino)alkylthio" means alkylthio as
defined herein, wherein an alkyl hydrogen atom is replaced
by an arylamino as defined herein. Examples of
(arylamino)alkylthio radicals include 2-(phenylamino)-
ethylthio, 3-(2-naphthylamino)-n-propylthio, and the like.

The term "(arylthio)alkylthio" means alkylthio as
defined herein, wherein an alkyl hydrogen atom is replaced
by an arylthio as defined herein. Examples of
(arylthio)alkylthio radicals include 2-(naphthylthio)-
ethylthio, 3-(phenylthio)-propylthio, and the like.

The term "heteroaryl" means a radical defined by an
aromatic heterocyclic ring as commonly understood in the
art, including monocyclic radicals such as, for example,
imidazole, thiazole, pyrazole, pyrrole, furane,
pyrazoline, thiophene, oxazole, isoxazol, pyridine,
pyridone, pyrimidine, pyrazine, and triazine radicals,
and also including polycyclics such as, for example,
quinoline, isoquinoline, indole, and benzothiazole
radicals, which heteroaryl radicals are optionally
substituted with one or more substituents selected from
the group consisting of a halogen, an alkyl, alkoxy,
amino, cyano, nitro, and the like. It will be appreciated
that the heterocycloalkyl and heteroaryl substituents can
be coupled to the compounds of the present invention via a
heteroatom, such as nitrogen (e.g., 1-imidazolyl).

The term "heteroaryloxy" means heteroaryl as defined
herein, wherein a hydrogen atom on the heteroaryl ring is
replaced by an oxygen. Heteroaryloxy radicals include,

44

for example, 4-pyridyloxy, 5-quinolyloxy, and the like.

The term "heteroarylamino" means heteroaryl as
defined herein, wherein a hydrogen atom on the heteroaryl
ring is replaced by an nitrogen. Heteroarylamino radicals

5   include, for example, 4-thiazolylamino, 2-pyridylamino,
and the like.

The term "heteroarylthio" means heteroaryl as defined
herein, wherein a hydrogen atom on the heteroaryl ring is
replaced by a sulfur. Heteroarylthio radicals include,

10   for example, 3-pyridylthio, 3-quinolylthio, 4-
imidazolylthio, and the like.

The term "heteroaralkyl" means alkyl as defined
herein, wherein an alkyl hydrogen atom is replaced by a
heteroaryl as defined herein. Examples of heteroaralkyl

15   radicals include 2-pyridylmethyl, 3-(4-thiazolyl)-propyl,
and the like.

The term "heteroaralkoxy" means alkoxy as defined
herein, wherein an alkyl hydrogen atom is replaced by a
heteroaryl as defined herein. Examples of heteroaralkoxy

20   radicals include 2-pyridylmethoxy, 4-(1-imidazolyl)-
butoxy, and the like.

The term "heteroaralkylamino" means alkylamino as
defined herein, wherein an alkyl hydrogen atom is replaced
by a heteroaryl as defined herein. Examples of

25   heteroaralkylamino radicals include 4-pyridylmethylamino,
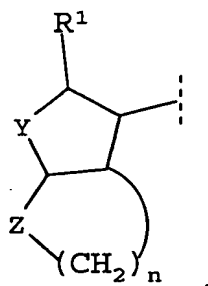3-(2-furanyl)-propylamino, and the like.

The term "heteroaralkylthio" means alkylthio as
defined herein, wherein an alkyl hydrogen atom is replaced
by a heteroaryl as defined herein. Examples of

30   heteroaralkylthio radicals include 3-pyridylmethylthio, 3-
(4-thiazolyl)-propylthio, and the like.

In the compound of Formula I, A is preferably a
group of the formula:

R¹ is H or an alkyl, an alkenyl, a cycloalkyl, a
cycloalkylalkyl, an aryl, an aralkyl, a heterocycloalkyl,
a heterocycloalkylalkyl, a heteroaryl, or a heteroaralkyl
radical, in which at least one hydrogen atom is
optionally substituted with a substituent independently
selected from the group consisting of OR^7, SR^7, CN, NO_2,
N_3, and a halogen, wherein R^7 is H, an unsubstituted
alkyl, or an unsubstituted alkenyl; Y and Z are the same
or different and are independently selected from the
group consisting of CH_2, O, S, SO, SO_2, NR^8, $R^8C(O)N$,
$R^8C(S)N$, $R^8OC(O)N$, $R^8OC(S)N$, $R^8SC(O)N$, $R^8R^9NC(O)N$, and
$R^8R^9NC(S)N$, wherein R^8 and R^9 are independently selected
from the group consisting of H, an unsubstituted alkyl,
and an unsubstituted alkenyl; X is a covalent bond, CHR^{10},
$CHR^{10}CH_2$, CH_2CHR^{10}, O, NR^{10}, or S, wherein R^{10} is H, an
unsubstituted alkyl, or an unsubstituted alkenyl; R^2 is
H, a C_1-C_6 alkyl radical, or a C_2-C_6 alkenyl radical; R^{12}
and R^{13}, as defined with respect to R^3, are independently
selected from the group consisting of H, an unsubstituted
alkyl, and an unsubstituted alkenyl radical; R^4 is OH,
NH_2, or $NHCH_3$; W is C(O), C(S), or SO_2; and R^6 is a
cycloalkyl, heterocycloalkyl, aryl, or heteroaryl radical
in which at least one hydrogen atom is optionally

substituted with a substituent independently selected from the group consisting of a halogen, OR^{15}, SR^{15}, CN, N_3, NO_2, $NR^{15}R^{16}$, $C(O)R^{15}$, $C(S)R^{15}$, CO_2R^{15}, $C(O)SR^{15}$, $C(O)NR^{15}R^{16}$, $C(S)NR^{15}R^{16}$, $NR^{15}C(O)R^{16}$, $NR^{15}C(S)R^{16}$, $NR^{15}CO_2R^{16}$, $NR^{15}C(O)SR^{16}$,

5       $NR^{15}C(O)NR^{16}R^{17}$, and $NR^{15}C(S)NR^{16}R^{17}$, an alkyl, an alkoxy, an alkylthio, an alkylamino, a cycloalkyl, a cycloalkylalkyl, a heterocycloalkyl, a heterocycloalkylalkyl, an aryl, an aryloxy, an arylamino, an arylthio, an aralkyl, an aryloxyalkyl, an

10   arylaminoalkyl, an aralkoxy, an (aryloxy)alkoxy, an (arylamino)alkoxy, an (arylthio)alkoxy, an aralkylamino, an (aryloxy)alkylamino, an (arylamino)alkylamino, an (arylthio)alkylamino, an aralkylthio, an (aryloxy)alkylthio, an (arylamino)alkylthio, an

15   (arylthio)alkylthio, a heteroaryl, a heteroaryloxy, a heteroarylamino, a heteroarylthio, a heteroaralkyl, a heteroaralkoxy, a heteroaralkylamino, and a heteroaralkylthio, wherein R^{15}, R^{16}, and R^{17} are H, an unsubstituted alkyl, and an unsubstituted alkenyl, such

20   that when at least one hydrogen atom of R^6 is optionally substituted with a substituent other than a halogen, OR^{15}, SR^{15}, CN, N_3, NO_2, $NR^{15}R^{16}$, $C(O)R^{15}$, $C(S)R^{15}$, CO_2R^{15}, $C(O)SR^{15}$, $C(O)NR^{15}R^{16}$, $C(S)NR^{15}R^{16}$, $NR^{15}C(O)R^{16}$, $NR^{15}C(S)R^{16}$, $NR^{15}CO_2R^{16}$, $NR^{15}C(O)SR^{16}$, $NR^{15}C(O)NR^{16}R^{17}$, or $NR^{15}C(S)NR^{16}R^{17}$, at least one

25   hydrogen atom on said substituent attached to R^6 is optionally substituted with a halogen, OR^{15}, SR^{15}, CN, N_3, NO_2, $NR^{15}R^{16}$, $C(O)R^{15}$, $C(S)R^{15}$, CO_2R^{15}, $C(O)SR^{15}$, $C(O)NR^{15}R^{16}$, $C(S)NR^{15}R^{16}$, $NR^{15}C(O)R^{15}$, $NR^{15}C(S)R^{16}$, $NR^{15}CO_2R^{16}$, $NR^{15}C(O)SR^{16}$, $NR^{15}C(O)NR^{16}R^{17}$, or $NR^{15}C(S)NR^{16}R^{17}$.

30   It is further preferred that when R^1 is an alkyl or an alkenyl radical (i.e., an alkyl or an alkenyl

substituent), then it is a C_1-C_6 alkyl or, in the case
when R^1 is an alkenyl, it is a C_2-C_6 alkenyl. When R^1 is a
monocyclic substituent such as, for example, a
cycloalkyl, a heterocycloalkyl, an aryl, or a heteroaryl,

5   it preferably comprises 4-7 members in the ring that
defines the monocyclic skeleton. When R^7, R^8 or R^9 is an
unsubstituted alkyl, it is preferably a C_1-C_6
unsubstituted alkyl; and when R^7, R^8 or R^9 is an
unsubstituted alkenyl, it is preferably a C_2-C_6

10 unsubstituted alkenyl. The ring defined by R^3 preferably
comprises 4-7 members or, in the case of polycyclics,
each ring comprises 4-7 members. When R^3 is $(CH_2)_pR^{11}$, the
ring defined by R^{11} preferably comprises 4-7 members, or,
in the case of polycyclics, each ring comprises 4-7

15 members. When either of R^{12} or R^{13} is an unsubstituted
alkyl, it is preferably a C_1-C_6 unsubstituted alkyl, and
when either of R^{12} or R^{13} is an unsubstituted alkenyl, it
is a C_2-C_6 unsubstituted alkyl. When R^{14} is a cycloalkyl,
a heterocycloalkyl, an aryl, or a heteroaryl, the ring

20 defined by R^{14} preferably comprises 4-7 members, or, in
the case of polycyclics, each ring comprises 4-7 members.
When R^6 is a cycloalkyl, a heterocycloalkyl, aryl, or a
heteroaryl, the ring defined by R^6 preferably comprises
4-7 members, or, in the case of polycyclics, each ring

25 comprises 4-7 members, and when R^6 is substituted with a
substituent that is an alkyl, an alkylthio, or an
alkylamino, it is preferred that the substituent
comprises from one to six carbon atoms, and when R^6 is
substituted with a substituent that is a cycloalkyl, a

30 heterocycloalkyl, an aryl, or a heteroaryl, the ring
defined by the substituent preferably comprises 4-7

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members or, in the case of polycyclics, each ring

comprises 4-7 members.

In a preferred embodiment, the method of preventing

the emergence of resistance in accordance with the

5   present invention includes administering a compound of

Formula (I), wherein Q is C(O), R^2 is H, and W is C(O) or

SO_2. In a further preferred embodiment, Q is C(O), R^2 is

H, R^4 is OH, W is SO_2, and the stereochemical orientation

of the asymmetric centers is represented by formula (IA)

10   or (IB) below:

(IA)   or

15   (IB).
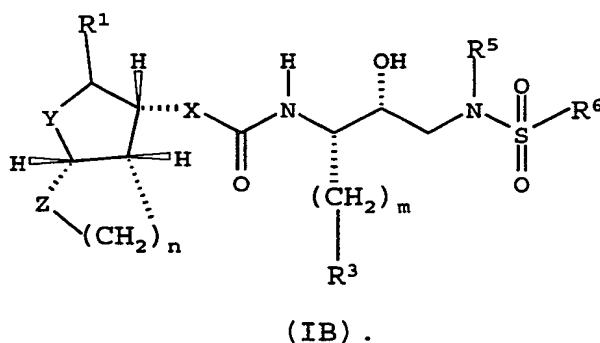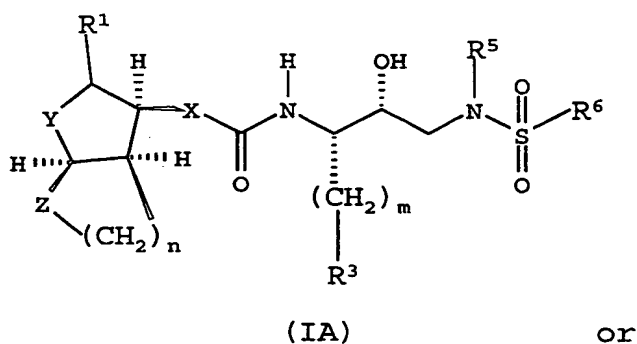
It is further preferred that R^6 is a monocyclic

substituent, preferably an aromatic ring, which is

preferably a substituted benzene ring, as illustrated by
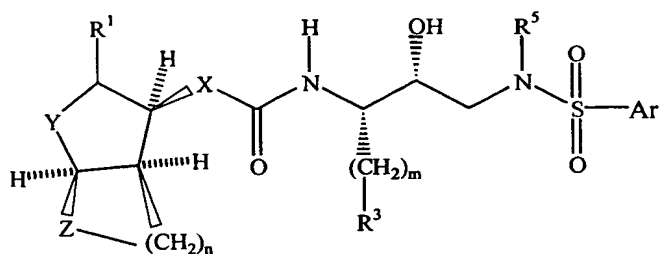
20   the formula:

49

(IC)   or
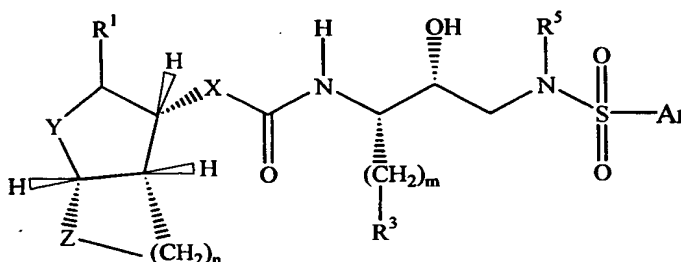
(ID),

wherein Ar is a phenyl which is optionally substituted with a substituent selected from the group consisting of methyl, amino, hydroxy, methoxy, methylthio, hydroxymethyl, aminomethyl, and methoxymethyl.

In a preferred series, Y and Z are oxygen atoms, n is 2, the resulting bis-tetrahydrofuranyl ring system has the stereochemical orientations illustrated in Formulae (1C) and (ID) above, m is 1, and R^3 is phenyl, in which case the compound is represented by the formula:
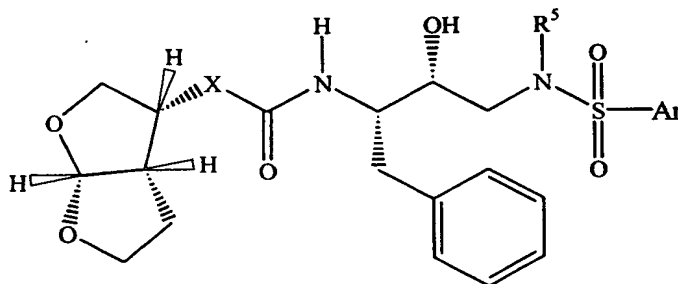
(IE)   or
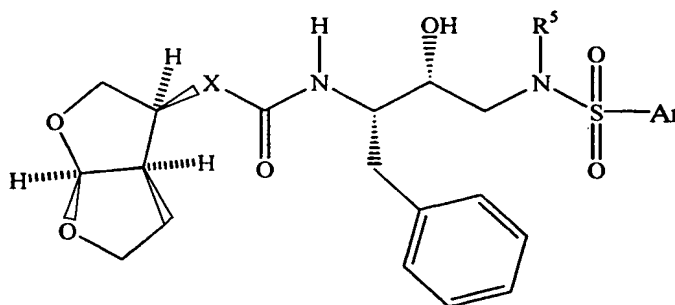
(IF),

wherein Ar is a phenyl which is optionally substituted
with a substituent selected from the group consisting of

5   methyl, amino, hydroxy, methoxy, methylthio,
hydroxymethyl, aminomethyl, and methoxymethyl. When the
compound is a compound of Formula (IE) or (IF), wherein
at least one hydrogen atom on Ar substituted with a
substituent selected from the group consisting of methyl,

10   amino, hydroxy, methoxy, methylthio, hydroxymethyl, and
methoxymethyl, it is further preferred that X is an
oxygen. Still more preferably, X is an oxygen and R^5 is
isobutyl. Suitable Ar substituents include phenyl groups
that are substituted at the para position, the meta

15   position, and/or the ortho position. Examples of
suitable Ar substituents are shown in Table 4, and in
Figures 3 and 5A-5D.

       A resistance-inhibiting effective amount is an amount
sufficient to produce an *in vivo* drug concentration or

20   level in which the biochemical vitality of a mutant HIV is
lower than the biochemical vitality of the HIV
(predecessor) infecting the HIV-infected mammal. For
example, a resistance-inhibiting effective amount is an
amount sufficient to produce an *in vivo* drug concentration

25   or level where the value for biochemical fitness is less
than one, when determined by the ratio of the biochemical

vitality of the mutant to the biochemical vitality of the
predecessor. The compound can be administered to a wild-
type HIV-infected mammal to prevent the emergence of first
line resistance, or it can be administered to a mammal

5   infected with a mutant-HIV to prevent the emergence of
drug resistance due to further mutations.

The compound is preferably administered in the form
of a pharmaceutical composition. The pharmaceutical
composition preferably includes a pharmaceutically

10   acceptable carrier and a resistance-inhibiting effective
amount of at least one of the aforesaid compound, alone or
in combination with another antiretroviral compound such
as, for example, a wild-type HIV protease inhibitor, a
mutant HIV retroviral protease inhibitor, or a reverse

15   transcriptase inhibitor. Generally, the pharmaceutical
composition of the present invention comprises a
resistance-inhibiting effective amount of at least one
compound of Formula (I), as disclosed herein, and a
pharmaceutically acceptable carrier.

20   In a preferred embodiment, a pharmaceutical
composition is administered that comprises a resistance-
inhibiting effective amount of at least one compound of
Formula (IA) or Formula (IB), or a pharmaceutically
acceptable salt, prodrug, or ester thereof, and a

25   pharmaceutically acceptable carrier. In a further
preferred embodiment, the pharmaceutical composition
comprises a resistance-inhibiting effective amount of at
least one compound of Formula (IC) or Formula (ID), or a
pharmaceutically acceptable salt, prodrug, or ester

30   thereof, and a pharmaceutically acceptable carrier. In a
highly preferred embodiment, the pharmaceutical

composition comprises a resistance-inhibiting effective
amount of at least one compound of Formula (IE), and
pharmaceutically acceptable salts, prodrugs, and esters
thereof, and a pharmaceutically acceptable carrier.

5   Pharmaceutically acceptable carriers are well-known
to those of skill in the art. The choice of a carrier
will be determined in part by the particular composition,
as well as by the particular mode of administration.
Accordingly, there are a wide variety of suitable
10   formulations for administration in accordance the present
invention.

        The pharmaceutical composition may be administered in
a form suitable for oral use such as, for example,
tablets, troches, lozenges, aqueous or oily suspensions or
15   solutions, dispersible powders or granules, emulsions,
hard or soft capsules, syrups or elixirs. Compositions
intended for oral use may be prepared according to any
method known in the art form the manufacture of
pharmaceutical compositions, and such compositions can
20   contain one or more agents such as, for example,
sweetening agents, flavoring agents, coloring agents, and
preserving agents in order to provide a pharmaceutically
elegant and/or palatable preparation. Tablets can contain
the active ingredient in admixture with non-toxic
25   pharmaceutically acceptable excipients which are suitable
for manufacture of tablets. Such excipients can be, for
example, inert diluents such as, for example, calcium
carbonate, lactose, calcium phosphate or sodium phosphate;
granulating and disintegrating agents such as, for
30   example, maize starch or alginic acid; binding agents such
as, for example, starch, gelatine or acacia, and

lubricating agents such as, for example, stearic acid or
talc. The tablets may be uncoated or they may be coated
by known techniques to delay disintegration and absorption
in the gastrointestinal tract and thereby provide a
5   sustained action over a longer period. For example, a
time delay material such as glyceryl monostearate or
glyceryl distearate alone or with a wax may be employed.

      Formulations for oral use also can be presented as
hard gelatin capsules wherein the active ingredient is
10   mixed with an inert solid diluent, for example calcium
carbonate, calcium phosphate or kaolin, or as soft gelatin
capsules wherein the active ingredient is mixed with water
or an oil medium, for example arachis oil, peanut oil,
liquid paraffin or olive oil.

15   Aqueous suspensions typically contain the active
materials in admixture with excipients suitable for the
manufacture of aqueous suspensions. Such excipients are
suspending agents, for example, sodium carboxymethyl
cellulose, methylcellulose, hydroxypropylmethylcellulose,
20   sodium alginate, polyvinylpyrrolidone, gum tragacanth and
gam acacia; dispersing or wetting agents may be a natural-
occurring phosphatide, for example, lecithin, or
condensation products of an alkylene oxide with fatty
acids, for example polyoxyethylene stearate, or
25   condensation products of ethylene oxide with long chain
aliphatic alcohols, for example
heptadecaethyleneoxycetanol, or condensation products of
ethylene oxide with partial esters derived from fatty
acids and a hexitol such as polyoxyethylene sorbitol
30   monooleate, or condensation products of ethylene oxide
with partial esters derived from fatty acids and hexitol

54

anhydrides, for example polyoxyethylene sorbitan mono-
oleate. The aqueous suspensions also can contain one or
more preservatives, for example, ethyl or n-propyl p-
hydroxy benzoate, one or more coloring agents, one or more
5   flavoring agents and one or more sweetening agents such
as, for example, sucrose or saccharin.

Oily suspensions may be formulated by suspending the
active ingredient in a vegetable oil, for example arachis
oil, olive oil, sesame oil or coconut oil, or in a mineral
10   oil such as liquid paraffin. The oil suspensions may
contain a thickening agent, for example beeswax, hard
paraffin or cetyl alcohol. Sweetening agents, such as
those set forth above, and flavoring agents may be added
to provide a palatable oral preparation. These
15   compositions can be preserved by the addition of an
antioxidant such as, for example, ascorbic acid.

Dispersible powders and granules suitable for
preparation of an aqueous suspension by the addition of
water provide the active ingredient in admixture with a
20   dispersing or wetting agent, suspending agent and one or
more preservatives. Suitable dispersing or wetting agents
and suspending agents are exemplified by those already
mentioned above. Additional excipients, for example
sweetening, flavoring and coloring agents, also may be
25   present.

The pharmaceutical composition also can be
administered in the form of oil-in-water emulsions. The
oily phase can be a vegetable oil, for example, olive oil
or arachis oils, or a mineral oil, for example liquid
30   paraffin or mixtures of these. Suitable emulsifying
agents may be naturally-occurring gums, for example gum

acacia or gum tragacantn, naturally-occurring

phosphatides, for example soya bean lecithin, and esters

or partial esters derived from fatty acids and hexitol

anhydrides, for example sorbitan mono-oleate, and

5   condensation products of the said partial esters and

ethylene oxide, for example polyoxyethylene sorbitan mono-

oleate.   The emulsions also can contain sweetening and

flavoring agents.

The pharmaceutical composition also can be

10   administered in the form of syrups and elixirs, which are

typically formulated with sweetening agents such as, for

example, glycerol, sorbitol or sucrose.   Such formulations

also can contain a demulcent, a preservative and flavoring

and coloring agents.

15   Further, the pharmaceutical composition can be

administered in the form of a sterile injectable

preparation, for example, as a sterile injectable aqueous

or oleagenous suspension.   Suitable suspensions for

parenteral administration can be formulated according to

20   the known art using those suitable dispersing or wetting

agents and suspending agents which have been mentioned

above.   Formulations suitable for parenteral

administration include, for example, aqueous and non-

aqueous, isotonic sterile injection solutions, which can

25   contain anti-oxidants, buffers, bacteriostates, and

solutes that render the formulation isotonic with the

blood of the intended recipient, and aqueous and non-

aqueous sterile suspensions that can include suspending

agents, solubilizers, thickening agents, stabilizers, and

30   preservatives.   The sterile injectable preparation can be

a solution or a suspension in a non-toxic parenterally-

acceptable diluent or solvent, for example, as a solution
in water or 1,3-butanediol. Among the acceptable vehicles
and solvents that can be employed, for example, are water,
Ringer's solution and isotonic sodium chloride solution.

5   In addition, sterile, fixed oils are conventionally
employed as a solvent or suspending medium. For this
purpose any bland fixed oil can be employed including
synthetic mono- or diglycerides. In addition, fatty acids
such as, for example, oleic acid find use in the

10 preparation of injectables.

Further, the compound can be administered in the form
of suppositories for rectal administration of the drug.
These compositions can be prepared by mixing the drug with
a suitable non-irritating excipient which is solid at

15 ordinary temperatures but liquid at the rectal temperature
and will therefore melt in the rectum to release the drug.
Such materials include, for example, cocoa butter and
polyethylene glycols. Formulations suitable for vaginal
administration can be presented as pessaries, tampons,

20 creams, gels, pastes, and foams.

Formulations suitable for topical administration may
be presented as creams, gels, pastes, or foams,
containing, in addition to the active ingredient, such
carriers as are known in the art to be appropriate.

25 The composition can be made into an aerosol
formulation to be administered via inhalation. Such
aerosol formulations can be placed into pressurized
acceptable propellants, such as dichlorodifluoromethane,
propane, nitrogen, and the like. They also can be

30 formulated as pharmaceuticals for non-pressured
preparations such as in a nebulizer or an atomizer.

The formulations can be presented in unit-dose or multi-dose sealed containers, such as ampules and vials, and can be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile

5   liquid excipient, for example, water, for injections, immediately prior to use. Extemporaneous injection solutions and suspensions can be prepared from sterile powders, granules, and tablets of the kind previously described.

10   Any suitable dosage level can be employed in the pharmaceutical compositions of the present invention. The dose administered to an animal, particularly a human, in the context of the present invention should be sufficient to effect a prophylactic or therapeutic response in the

15 animal over a reasonable time frame. The amount of active ingredient that can be combined with the carrier materials to produce a single dosage form will vary depending upon the host treated and the particular mode of administration. The size of the dose also will be

20 determined by the existence, nature, and extent of any adverse side-effects that might accompany the administration of a particular composition. Suitable doses and dosage regimens for the prevention of drug resistance can be determined by comparisons to

25 antiretroviral chemotherapeutic agents that are known to inhibit the proliferation of a retrovirus in an infected individual. The preferred dosage is the amount that results in the inhibition of the emergence of mutant drug-resistant retroviruses, particularly the emergence of

30 multidrug-resistant retroviral HIV, without significant side effects. In proper doses and with suitable

administration of certain compounds, a wide range of
antiretroviral chemotherapeutic compositions are possible.
A suitable dose includes a dose or dosage which would be
insufficient to completely suppress the growth of a wild-
5   type or predecessor virus, but would be sufficient to
inhibit or effectively suppress the growth of a mutant.

In accordance with the present invention, the
compound or composition can be administered in combination
with other antiretroviral compounds such as, for example,
10   ritonavir, amprenavir, saquinavir, indinavir, AZT, ddI,
ddC, D4T, lamivudine, 3TC, and the like, as well as
admixtures and combinations thereof, in a pharmaceutically
acceptable carrier. The individual daily dosages for
these combinations can range from about one-fifth of the
15   minimally recommended clinical dosages to the maximum
recommended levels for the entities when they are given
singly.

The present invention also provides a method of
preventing the emergence of multidrug-resistant
20   retroviruses in an HIV-infected mammal, which method
comprises administering to the mammal a multidrug
resistance-inhibiting effective amount of a compound of
the present invention, so as to inhibit the emergence of
a multidrug-resistant retrovirus in the mammal. The dose
25   administered to an animal, particularly a human in the
context of the present invention, should be sufficient to
effect a therapeutic response in the animal over a
reasonable time frame. The dose will be determined by the
strength of the particular composition employed and the
30   condition of the animal, as well as the body weight of the
animal to be treated. The size of the dose will also be

determined by the existence, nature, and extent of any
adverse side-effects that might accompany the
administration of a particular compound. Other factors
which effect the specific dosage include, for example,

5   bioavailability, metabolic profile, and the
pharmacodynamics associated with the particular compound
to be administered in a particular patient. One skilled
in the art will recognize that the specific dosage level
for any particular patient will depend upon a variety of

10   factors including, for example, the activity of the
specific compound employed, the age, body weight, general
health, sex, diet, time of administration, route of
administration, rate of excretion, drug combination, CD4
count, the potency of the active compound with respect to

15   the particular mutant retroviral strain to be inhibited,
and the severity of the symptoms presented prior to or
during the course of therapy. What constitutes a
resistance-inhibiting effective amount can be determined,
in part, by use of one or more of the assays described

20   herein, particularly the fitness assay of the present
invention.

One skilled in the art will appreciate that suitable
methods of administering compounds and pharmaceutical
compositions are available, and, although more than one

25   route can be used to administer a particular composition,
a particular route can provide a more immediate and/or
more effective reaction than another route.

Numerous compounds have been identified that exhibit
potent antiretroviral activity, in particular retroviral

30   protease activity, against wild-type HIV. However, among
the fifteen currently FDA-approved antiretroviral agents

60

which are all known potent inhibitors of wild-type HIV,
five of which are potent inhibitors of wild-type HIV
protease, none of these compounds have the ability to
prevent the emergence of drug-resistance mutations that

5   are associated with high level cross resistance. Thus,
these inhibitors do not have the ability to suppress the
sufficiently fit mutant retroviruses that can (and almost
certainly will) emerge under the selection pressure of
these inhibitors.

10   Surprisingly, it has been discovered that compound
32 (shown in Figure 3A), which is a potent wild-type HIV
inhibitor, possesses remarkably potent and unprecedented
broad-spectrum inhibitory activity against a panel of
recombinant mutant HIV protease targets. These enzymes

15   represent the key or primary resistance mutations, most
of which occur in the active site region. Based on this
finding, the compound was tested against a panel of drug
resistant mutant patient isolates of HIV and was found to
possess broad spectrum antiviral activity against a wide

20   range of clinically isolated, multiply drug-resistant,
human immunodeficiency viruses. Other compounds
described herein showed similar activity. The mutant
viruses were obtained from infected humans who had
received several antiviral drugs. Although applicants do

25   not wish to abound by any one particular theory, it is
believed that the combination of the bicyclic ligand
(vii) with isostere (vi) gives the antiretroviral
compounds of the present invention the unique ability to
bind to the active site of the mutant proteases of

30   multiply drug-resistant human immunodeficiency viruses
generally, which trait has heretofore not been reported

with respect to any known chemotherapeutic and/or
experimental HIV protease inhibitor. A wild-type
preliminary screen was utilized to determine the
antiretroviral activity of analogs against wild-type HIV.

5   It is predicted that compounds of Formula (I), which have
potent antiretroviral or protease-inhibitory activity
against wild-type HIV, also will be potent inhibitors of
drug-resistance, even multiple drug-resistance, in wild-
type HIV, or even a mutant thereof.

10   The resistance-inhibiting compounds of the present
invention can be synthesized by any suitable method known in
the art. The preferred synthesis method is generally
illustrated in Figure 4, which is an representation of the
synthetic approach to preparing a preferred series of

15   compounds, wherein a compound of Formula (I) is synthesized
in several steps starting from azidoepoxide (i), wherein R^1-
R^{17}, m, n, p, Q, W, X, y, and z are defined as above.
Referring to Figure 4, amine (ii) is nucleophilically added
to azidoepoxide (i), providing aminoalcohol (iii). The

20   amine functional group of aminoalcohol (iii) is then reacted
with intermediate (iv), wherein L represents a leaving group
(e.g., halogen, N-oxysuccinimide), which can be displaced by
the amine of aminoalcohol (iii), to provide azide (v).
Reduction of azide (v), or, when R^5 is not hydrogen,

25   reductive amination with aldehyde $R^5CH=O$, provides
intermediate (vi), which is subsequently coupled with
activated bicyclic ligand (vii), to provide compounds of
Formula I. Of course, it will be appreciated by a person of
·ordinary skill in the art that there are combinations of

30   substituents, functional groups, R-groups, and the like,
which are reactive under particular reaction conditions, and
require the utilization of an appropriate protecting group

or groups, which are known in the art, to ensure that the desired synthetic transformation will take place without the occurrence of undesired side reactions. For example, possible substituents at R^5 (e.g., NH_2) can be competitive

5   nucleophiles requiring the attachment of an appropriate protecting group thereon (e.g., benzyloxycarbonyl, tert-butoxycarbonyl) in order obtain proper selectivity in the ring opening of epoxide (i) with amine (ii).

Figures 1-3B illustrate the synthesis of a preferred

10   series of compounds for use in the method of preventing the emergence of resistance in accordance with the present invention. Figure 1, which is a synthetic scheme for the synthesis of a particular sulfonamide, illustrates the synthesis of a preferred isosteric core, particularly, the

15   sulfonamide isosteric core represented by aminosulfonamide 15. With reference to Figure 1, aminosulfonamide core 15 can be synthesized by initially providing azidoepoxide 11 and subjecting it to nucleophilic addition with amine 12 to give aminoalcohol 13, which is subsequently converted

20   to sulfonamide 14 by reaction with 4-methoxybenzenesulfonyl chloride. The azide group of 14 is then reduced to provide aminosulfonamide 15, which can be used as a core for synthesizing numerous multidrug-resistant retroviral protease inhibitors of the present

25   invention.

Figure 2, which is a reaction scheme detailing the preparation of bicyclic alcohols, illustrates the synthesis of a preferred series of bicyclic ligands, particularly bis-tetrahydrofurans 25 and 26. With

30   reference to Figure 2, dihydrofuran 21 is treated with N-iodosuccinimide in the presence of propargyl alcohol to

give iodoether 22, which is cyclized to methylene-
substituted bis-tetrahydrofuran 23. Ozonolysis of the
exo-methylene residue of 23, followed by reduction,
provides bicyclic racemic alcohol 24, which is resolved to

5   give, separately, bicyclic alcohol 25 and its enantiomeric
acetate ester 26, which ester group of 26 is subsequently
hydrolyzed to afford enantiomer 27.

Figures 3A and 3B, which are reaction schemes
describing the preparation of two protease inhibitors,

10   illustrate the preparation of two preferred multidrug-
resistant HIV protease inhibitors of the present
invention. With reference to Figure 3A, compound 32 was
synthesized by coupling succinimidocarbonate 31 with
aminosulfonamide 15. Succinimidocarbonate 31 was prepared

15   by reacting optically pure bicyclic alcohol 25 with
disuccinimidyl carbonate in the presence of triethylamine.
Inhibitor 34, which possesses the enantiomeric bis-
tetrahydrofuranyl ligand (relative to inhibitor 32), was
prepared in the same fashion, except that the enantiomeric

20   bicyclic alcohol 27 was used instead of alcohol 25, as
illustrated in Figure 3B.

The following examples further illustrate the present
invention but, of course, should not be construed as in

25   any way limiting its scope.

Example 1
This example describes the synthesis of exemplary
epoxide 11 (Figure 1), which is used as an intermediate

30   in the synthesis of a particular series of compounds
within the scope of the present invention.

64

Anhydrous CuCN (4.86 g, 54 mmol) was added to a solution of butadiene monooxide (38 g, 540 mmol) in anhydrous tetrahydrofuran (1.2 L) and the resulting mixture was stirred at -78°C. Commercial phenyl

5   magnesium bromide solution (Aldrich) in ether (65 mmol) was added dropwise over a period of 10 min. The resulting reaction mixture was then allowed to warm to 0 °C and it was continued to stir until the reaction mixture was homogeneous. After this period, the reaction

10   mixture was cooled to -78 °C and 0.58 mole of phenylmagnesium bromide solution in ether was added dropwise for 30 min. The reaction mixture was allowed to warm to 23 °C for 1 h. The reaction was quenched by slow addition of saturated aqueous NH_4Cl (120 mL) followed by

15   NH_4OH (70 mL), saturated NH_4Cl (500 ML) and then H_2O (300 mL). The aqueous layer was thoroughly extracted with ethyl acetate (2 x 300 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was

20   distilled under vacuum (0.12 torr) at 95 °C to give trans-4-phenyl-2-butene-1-ol (75.6 g).

To a suspension of powdered 4Å molecular sieves (6.6 g) in anhydrous methylene chloride (750 mL), titanium tetraisopropoxide (Aldrich, 3.2 mL) and then diethyl D-

25   tartrate (2.3 mL) were added. The resulting mixture was cooled to -22 °C and tert-butylhydroperoxide solution in isooctane (Aldrich, 430 mmol) was added over a period of 10 min. The mixture was stirred an additional 30 min and then a solution of trans-4-phenyl-2-butene-1-ol (32.6 g,

30   213 mmol), in anhydrous methylene chloride (120 mL), was added dropwise over a period of 40 min at -22 °C. The

reaction mixture was then aged in a freezer at -22 °C for
24 h. After this period, water (100 mL) was added to the
reaction mixture at -22 °C and the mixture was allowed to
warm to 0 °C. After stirring at 0 °C for 45 min, 20% NaOH
5   in brine (20 mL) was added. The resulting mixture was
then allowed to warm to 23 °C and was stirred at that
temperature for 1 h. After this period, the layers were
separated and the aqueous layer was extracted with
methylene chloride (2 x 200 mL). The combined organic
10 layers were dried over anhydrous Na_2SO_4 and concentrated
under reduced pressure. The residue was diluted with
toluene (800 mL) and then evaporated under reduced
pressure. The residue was chromatographed over silica
gel (35% ethyl acetate in hexane as eluent) to provide
15 (2R, 3R)-epoxy-4-phenylbutan-1-ol (21.8 g).

To a solution of titanium ispropoxide (12 mL) in
anhydrous benzene (250 mL) was added azidotrimethylsilane
(11 mL) and the resulting mixture was refluxed for 6 h.
A solution of (2R,3R)-epoxy-4-phenylbutan-1-ol (5.32 g)
20 in anhydrous benzene (25 mL) was added to the above
refluxing mixture. The resulting mixture was refluxed
for addition 25 min. After this period, the reaction
mixture was cooled to 23 °C and the reaction was quenched
with aqueous 5% H_2SO_4 (400 mL). The resulting mixture was
25 stirred for 1 h and the layers were separated and the
aqueous layer was extracted with ethyl acetate (2 x 300
mL). The combined organic layers were washed with
saturated $NaHCO_3$ (200 mL), dried over Na_2SO_4 and
concentrated under reduced pressure to afford the
30 (2S,3S)-2-hydroxy-3-azido-4-phenyl-butan-12-ol (5.1 g) as
a white solid (mp 81-82 °C).

To a stirred solution of the azidodiol (5.1 g) in
chloroform (100 mL) at 23 °C, 2-acetoxyisobutyryl
chloride (Aldrich, 5mL) was added. The resulting
reaction mixture was stirred at 23 °C for 8 h. The

5   reaction was quenched by addition of saturated sodium
bicarbonate (100 mL) and the resulting mixture was
stirred 30 min. The layers were separated and the
aqueous layer was extracted with chloroform (2 x 200 mL).
The combined organic layer was extracted with chloroform

10   (2 x 200 mL). The combined organic layers were dried
over Na_2SO_4 and evaporated under reduced pressure. The
resulting residue was dissolved in anhydrous THF (50 mL)
and solid NaOMe (2.1 g) was added. The mixture was
stirred for 4 h at 23 °C and after this period, the

15   reaction was quenched with saturated NH_4Cl (50 mL). The
resulting mixture was extracted with ethyl acetate (2 x
200 ML). The combined organic layers were dried over
anhydrous Na_2SO_4 and concentrated under reduced pressure
to give a residue, which was chromatographed over silica

20   gel (10% ethyl acetate in hexanes) to afford the 3(S)-
azido-(1,2R)-epoxy-4-phenylbutane 11 (3.3 g) as an oil:
^{1}H NMR (300 MHz): $CDCl_3$; δ 7.4-7.2 (m, 5H,), 3.6 (m, 1H),
3.1 (m, 1H), 2.95 (dd, 1H, J = 4.6, 13.9 Hz), 2.8 (m,
3H).

25

Example 2
    This example illustrates the synthesis of
azidoalcohol 13 (Figure 1), which can be used as an
intermediate in the synthesis of a preferred series of

30   the compounds of the present invention.

To a stirred solution of above azidoepoxide 11 (700
mg, 3.7 mmol) in ispropanol (70 mL) was added isobutyl
amine (Aldrich, 0.74 mL 7.4 mmol) and the resulting
mixture was heated at 80 °C for 12 h. After this period,
5   the reaction mixture was concentrated under reduced
pressure and the residue was chromatographed over silica
gel to provide azidoalcohol 13 (800 mg) as an oil.

Example 3

10   This example illustrates the synthesis of
azidosulfonamide 14, the structure of which is shown in
Figure 1.

To a stirred solution of 13 (600 mg, 2.28 mmol) in
CH_2Cl_2 (20 mL) was added 4-methoxybenzenesulfonyl chloride
15   (Aldrich, 530 mg, 2.52 mmol) and saturated aqueous $NaHCO_3$
(6 mL). The resulting heterogeneous mixture was stirred
at 23 °C for 12 h. The reaction was diluted with CH_2Cl_2
and the layers were separated. The organic layer was
washed with brine, dried over anhydrous magnesium sulfate
20   and concentrated to dryness. The residue was
chromatographed over silica gel (25% ethyl
acetate/hexane) to provide 900 mg of azidosulfonamide 14.

Example 4

25   This example illustrates the preparation of
aminosulfonamide 15 via reduction of azidosulfonamide 14,
as shown in Figure 1.

A solution of 14 (1.53 g) in THF (45 mL), MeOH (10
mL) and acetic acid (0.5 mL), was shaken with 10%
30   palladium on carbon catalyst (200 mg) at 50 psi hydrogen
pressure for 2 h. Removal of the catalyst by filtration

over celite and concentration under reduced pressure gave

a crude residue, which was diluted with CH_2Cl_2 (100 mL),

and was washed successively with saturated aqueous $NaHCO_3$

and brine. The organic layer was dried over $MgSO_4$ and

5   concentrated to give the corresponding aminosulfonamide

15 (1.2 g).

Example 5

This example demonstrates the synthesis of trans-2-

10   (propargyloxy)-3-iodotetrahydrofuran 22 (Figure 2).

To a stirred, ice-cold suspension of 15 g (66.6

mmol) of N-iodosuccinimide in 150 mL of CH_2Cl_2 was added a

mixture of dihydrofuran 21 (66.6 mmol, 4.67 g, 5.1 mL)

and propargyl alcohol (100 mmol, 5.0 g, 5.2 mL) of in 50

15   mL of CH_2Cl_2 over 20 min. After warming to 24 °C with

stirring over 2 h, 200 mL of water were added and the

stirring continued for 1 h. The layers were separated

and the aqueous layer was extracted with 2 x 100 mL of

CH_2Cl_2. The combined organic extracts were washed with

20   brine solution containing small amount of $Na_2S_2O_3$ (70 mg),

dried over anhydrous Na_2SO_4, filtered, and concentrated.

Chromatography over silica gel using 30% ethyl acetate in

hexane afforded (15.4 g, 92%) the title iodoether 22 as

an oil.

25

Example 6

This example illustrates the synthesis of (±)-(3aR,

6aS) and (3aS, 6aR)-3-methylene-4H-hexahydrofuro-[2,3-

b]furan 23, as shown in Figure 2.

30   To a refluxing solution of (20.7 mL, 77 mmol)

tributyltin hydride containing AIBN (100 mg) in toluene

(200 mL) was added dropwise a solution of 15.4 g (61

mmol) of iodotetrahydrofuran 22 in toluene (50 mL) over a

period of 1 h. The resulting mixture was stirred at

reflux for an additional 4 h (monitored by TLC). The

5   mixture was then cooled to 23 °C and concentrated under

reduced pressure. The residue was partitioned between

petroleum ether and acetonitrile (200 mL of each) and the

acetonitrile (lower) layer was concentrated. The residue

was purified by chromatography on silica gel, using 10%

10   ethyl acetate in hexane as the eluent to provide the

title product 23 (5.84 g, 76%) as an oil.

Example 7

       This example demonstrates the synthesis of (±)-(3SR,

15   3aRS, 6aS) and (3R, 3aS, 6aR)-3-hydroxy-4H-

hexahydrofuro[2,3-b]furan 24, as shown in Figure 2.

       A stream of ozone was dispersed into a solution of

15 (5.84 g, 46.4 mmol) at -78 °C in 150 mL of methanol

and 150 mL of $CH_2Cl_2$for 30 min. The resulting blue

20   solution was purged with nitrogen until colorless, then

quenched with 20 mL of dimethyl sulfide and the resulting

mixture was allowed to warm to 23 °C. The mixture was

concentrated under reduced pressure to afford the crude

ketone. The resulting crude ketone was dissolved in

25   ethanol (50 mL) and the solution was cooled to 0 °C and

sodium borohydride (2.1 g, 55.6 mmol) was added. The

reaction mixture was stirred for an additional 2 h at 0

°C and then quenched with 10% aqueous citric acid (10

mL). The resulting mixture was concentrated under

30   reduced pressure and the reside was partitioned between

ethyl acetate and brine. The layers were separated and

the aqueous layer was extracted with ethyl acetate (2 x
100 mL). The combined organic layers were dried over
anhydrous-Na_2SO_4 and concentrated carefully under reduced
pressure. The resulting residue was chromatographed over

5   silica gel using 30% ethyl acetate in hexane as the
eluent to furnish (4.52 g, 75%) the title racemic alcohol
24 as an oil.

Example 8

10   This example illustrates the preparation of
immobilized Amano Lipase 30, which was used to resolve
racemic aminoalcohol 24 (Figure 2).

         Commercially available 4 g of Celite° 521 (Aldrich)
was loaded on a buchner funnel and washed successively

15   with 50 mL of deionized water and 50 mL of 0.05 N
phosphate buffer (pH = 7.0; Fisher Scientific). The
washed celite was then added to a suspension of 1 g of
Amano lipase 30 in 20 mL of 0.05 N phosphate buffer. The
resulting slurry was spread on a glass dish and allowed

20   to dry in the air at 23 °C for 48 h (weight 5.4 g; water
content about 2% by Fisher method).

71

Example 9

  This example demonstrates the synthesis of (3R,3aS, 6aR) 3-hydroxyhexahydrofuro[2,3-b]furan 25 by immobilized lipase catalyzed acylation, as illustrated in Figure 2.

5 To a stirred solution of reacemic alcohol 24 (2 g, 15.4 mmol) and acetic anhydride (4 g, 42.4 mmol) in 100 mL of DME was added 2.7 g (about 25% by weight of lipae PS30) of immobilized Amano lipase and the resulting suspension was stirred at 23 °C. The reaction was
10 monitored by TLC and ^{1}H NMR analysis until 50% conversion was reached. The reaction mixture was filtered and the filter cake was washed repeatedly with ehtyl acetate. The combined filtrate was carefully concentrated in a rotary evaporator, keeping the bath temperature below 15
15 °C. The residue was chromatographed over silica gel to provide 843 mg (42%) of 25 (95% ee; $a_D^{23°}$ -11.9°, MeOH); ^{1}H-NMR (CDCl$_3$) d 1.85 (m, 2H), 2.3 (m, 1H), 2.9 (m, 1H), 3.65 (dd, J=7.0, 9.1, 1H), 3.85-4.0(m, 3H), 4.45 (dd, J=6.8, 14.6, 1H), 5.7 (d, J=5.1, 1H); also, 1.21 g of 26
20 after washing with 5% aqueous sodium carbonate (45%, $a_D^{23°}$ +31.8°, MeOH); ^{1}H-NMR (CDCl$_3$)d 1.85-2.1 (m, 2H), 2.1 (s, 3H), 3.1 (m, 1H), 3.75(dd, J=6.6, 9.2, 1H), 3.8-4.1 (m, 3H), 5.2 (dd, J=6.4, 14.5, 1H), 5.7 (d, J=5.2, 1H). Acetate 26 was dissolved in THF (5mL) and 1 M aqueous
25 LiOH solution (20 mL) was added to it. The resulting mixture was stirred at 23°C for 3 h and the reaction was extracted with chloroform (3 x 25 mL). The combined organic layers were dried over anhydrous Na$_2$SO$_4$ and evaporated under reduced pressure. The residue was
30 chromatographed over silica gel to provide 733 mg of 27 (97% ee; $\alpha_D^{23°}$ -12.5°, MeOH).

Example 10

This example demonstrates the synthesis of activated carbonates 31 and 33, as illustrated in Figures 3A and

5   3B.

To a stirred solution of [3R,3aS, 6aS]-3-hydroxyhexahydrofuro[2,3-b]furan 25 (65 mg, 0.5 mmol) in dry CH₃CN (5 mL) at 23°C were added disuccinimidyl carbonate (192 mg, 0.75 mmol) and triethylamine (0.25

10   mL). The resulting mixture was stirred at 23°C for 12 h. The reaction was quenched with saturated aqueous NaHCO₃ (10 mL) and the mixture was concentrated under reduced pressure. The residue was extracted with CH₂Cl₂ (2 x 25 mL) and the combined organic layers were washed with

15   brine (10 mL) and dried over anhydrous Na₂SO₄. Evaporation of the solvent under reduced pressure gave a residue, which was chromatographed over silica gel (50% ethyl acetate/hexane) to furnish (3R, 3aS, 6aR) 3-hydroxyhexahydrofuro[2,3-b]furanyl-succinimidyl carbonate

20   31 (70 mg) as a brown oil. Carbonate 33 (65 mg) was prepared from 60 mg of alcohol 27 by following a similar procedure.

Example 11

25   This example illustrates the preparation of multidrug-resistant HIV inhibitor 32, as illustrated in Figure 3A.

To a stirred solution of amine 15 (82 mg, 0.2 mmol) in dry CH₂Cl₂ (5 mL) was added succinimidyl carbonate 31

30   (55 mg, 0.18 mmol). The resulting solution was stirred at 23°C for 12 h. After this period, the reaction was

quenched with saturated aqueous NaHCO$_3$ (10 mL) and diluted with CH$_2$Cl$_2$ (25 mL). The layers were separated and the organic layer was washed with brine (15 mL) and dried over anhydrous Na$_2$SO$_4$. Evaporation of the solvent

5 under reduced pressure afforded a residue, which was purified by silica gel chromatography (75% ethyl acetate/hexane) to furnish compound 32 (85 mg) as a white solid (m.p 55-58°C). ^{1}H-NMR (CDCl$_3$, 400 MHz); δ 7.71(d, 2H, J=8.8 Hz), 7.29-7.20 (m, 5H), 6.99 (d,2H,J=7.0 Hz),

10 5.65 (d,1H,J=5.19), 5.01 (m, 2H), 3.95-3.82 (m, 7H), 3.69 (m,2H), 3.0-2.7 (m, 6H), 1.85 (m, 1H), 1.64-1.45 (m, 3H), 0.90 (two d, 6H, J=6.5Hz, 6.6 Hz).

Example 12

15   This example illustrates the preparation of multidrug-resistant HIV inhibitor 33, as illustrated in Figure 3B.

     Carbonate 33 (55 mg) was reacted with amine 15 (82 mg, 0.2 mmol) according to the procedure mentioned above

20 to provide compound 34 (81 mg). ^{1}H-NMR (CDCl$_3$, 300 MHz); δ 7.69(d, 2H, J=8.8 Hz), 7.28-7.21 (m,5H), 6.87 (d,2H,J=5.84 Hz), 5.67 (d,1H,J=5.46 Hz), 5.0 (m, 2H), 3.86-3.81 (m, 7H), 3.58 (dd, 2H, J=6.6 Hz, 3.6 Hz, 3.17-2.73 (m, 6H), 2.17-1.83 (m, 4H), 0.90 (two d, 6H,

25 J=6.5Hz, 6.6 Hz).

Example 13

        This example describes the protocol for the
sensitive continuous fluorogenic assay for HIV protease
of the present invention and its application. Using this
5   assay, the inhibitory activity of compound 32 (Fig. 3A)
was tested against the proteases of wild-type HIV-1 (WT)
and various mutant enzynes: D30N, V32I, I84V, V32I/I84V,
M46F/V82A, G48V/L90M, V82F/I84V, V82T/I84V,
V32I/K45I/F53L/A71V/I84V/L89M,
10 V32I/L33F/K45I/F53L/A71V/I84V, and 20R/36I/54V/71V/82T,
which protease enzymes are available from Dr. John W.
Erickson, Structural Biochemistry Program, SAIC
Frederick, P.O. Box B, Frederick, MD 21702-1201, upon
written request. The inhibition constant for wild-type
15 HIV-1 , K_{imnt}/K_{iwt} ratio, and vitality were measured. (See
Gulnik et al., Biochemistry, 34, 9282-9287 (1995).
Protease activity was measured using the fluorgenic
substrate Lys-Ala-Arg-Val-Tyr-Phe(NO_2)-Glu-Ala-Nle-NH_2
(Bachem Bioscience, Inc.). (See Peranteau et al., D.H.
20 (1995) Anal. Biochem.).

        Typically, 490 μl of 0.125 M ACES-NaOH buffer, pH
6.2, containing 1.25 M $(NH_4)_2SO_4$, 6.25 mM DTT and 0.1%
PEG-8000 was mixed with 5 μl of titrated protease (final
concentration 1-5 nM) and incubated 3 min at 37 °C. The
25 reaction was initiated by the addition of 5 μl of
substrate stock solution in water. Increase in
fluorescence intensity at the emission maximum of 306 nm
(excitation wavelength was 277 nm) was monitored as a
function of time using Aminco Bowman-2 luminescence
30 spectrometer (SLM Instruments, Inc.). The initial rate
of hydrolysis was calculated by second degree polynomial

fit using SLM AB2 2.0 operating software. Kinetic

parameters were determined by nonlinear regression-

fitting of initial rate versus substrate concentration

data to the Michaelis-Menten equation using program

5   Enzfiter version 1.05.

For inhibition studies, inhibitors were prepared as

stock solutions at different concentrations in

dimethylsulfoxide. In a typical experiment 485 µl of

0.125 M ACES-NaOH buffer, pH 6.2, containing 1.25 M

10 $(NH_4)_2SO_4$, 6.25 mM DTT AND 0.1% PEG-8000, was mixed with 5

µl of inhibitor stock solution and 5 µl of titrated

protease (final concentration of 1-5 nM) and preincubated

3 min at 37 °C. The reaction was initiated by the

addition of 5 µl of substrate stock solution in water.

15 For data analysis, the mathematical model for tight-

binding inhibitors was used. (*See* Williams and Morrison

(1979), In: Methods of Enzymol. 63, (ed. D.L. Purich),

437-467, Academic Press, NY, London). The data were

fitted by nonlinear regression analysis to the equation:

20 $V=V_0/2E_t(\{[K_i(1+S/K_m)+I_t-E_t]^2+4K_i(1+S/K_m)E_t\}^{1/2}-[K_i((1+S/K_m)+I_t-E_t])$ with the program Enzfiter (version 1.05), where V

and V_0 are initial velocities with and without inhibitor,

respectively, K_m is a Michaelis-Menten constant, and S, E_t

and I_t are the concentrations of substrate, active

25 enzyme, and inhibitor, respectively. Biochemical fitness

for each mutant was determined by comparing the

biochemical vitality of each mutant (vitality$_{mut}$) with the

biochemical vitality of the wild-type reference

(vitality$_{wt}$), according to the formula

30   (vitality$_{mut}$)/(vitality$_{wt}$),

wherein vitality is $(K_i)(k_{cat}/K_M)$. The results are shown below in Table 1.

Table 1

Compound 32			
Enzyme	K_i (pM)	K_{I-mut}/K_{I-wt}	Biochemical Fitness
WT	14	1	1
D30N	<5	0.33	0.3
V32I	8	0.57	0.5
I84V	40	2.85	1
V32I/I84V	70	5	0.7
M46F/V82A	<5	0.33	0.1
G48V/L90M	<5	0.33	0.1
V82F/I84V	7	0.5	0.1
V82T/I84V	22	1.57	0.1
V32I/K45I/F53L/A71V/I84V/L89M	31	2.2	0.1
V32I/L33F/K45I/F53L/A71V/I84V	46	3.3	0.1
20R/36I/54V/71V/82T	31	2.2	0.1

5   The above results demonstrate that compound 32 is a potent inhibitor of multiple HIV protease mutants that contain the primary or key drug resistance mutations. These data predict that compound 32 will have potent and broad-spectrum multidrug-resistant antiretroviral

10   activity. Moreover, the biochemical fitness of each mutant relative to wild type is equal to or less than one in the presence of compound 32. Based on this fitness profile, it is believed that drug resistant viruses containing the characteristic mutations assayed herein

15   will not emerge from the wild-type in the presence of compound 32.

Example 14

This example illustrates the potent and broad-spectrum multidrug-resistant antiretroviral activity of an exemplary compound of the present invention.

Compound 32, shown in Figure 3A, was tested side-by-side with four other known HIV-1 protease inhibitors against various wild-type HIV-1 strains (HIV-1$_{ERS104pre}$, HIV-1$_{LAI}$, and HIV-1$_{BAL}$), and mutant multidrug-resistant HIV-1 strains clinically isolated from eight different patients who had received numerous antiviral drugs, either singly or in combination. The patients from which the mutant strains were isolated had a history of anti-HIV therapy with a variety of different drugs such as, for example, ritonavir, saquinavir, indinavir, amprenavir, AZT, ddI, ddC, d4T, 3TC, ABV (abacavir), DLV (delaviridine), and PFA (foscarnet). The patient profiles are shown below in Table 2.

Table 2

Patient/ Isolate Code	CD4$^+$ (/mm^3)	HIV-1 RNA level (copies/mL)	Months on Antiviral Therapy	Prior and Present Anti-HIV Therapy
1	361	246,700	64	AZT, ddI, ddC, d4T, 3TC, ABV, IDV, RTV, SQV, AMV, DLV
2	3	553,700	46	AZT, ddI, ddC, d4T, 3TC, ABV, IDV, SQV, AMV
3	108	42,610	39	AZT, ddI, ddC, d4T, 3TC, ABV, IDV, SQV, AMV
4	560	60,000	81	AZT, ddI, ddC, U90, d4T, 3TC, ABV, IDV, SQV, AMV
5	-	-	32	AZT, ddI, ddC, d4T, 3TC, ABV, IDV, SQV, AMV
6	-	-	34	AZT, ddI, ddC, d4T, 3TC, ABV, IDV, SQV, AMV
7	-	-	83	AZT, ddI, ddC, d4T, 3TC, ABV, IDV, SQV, RTV, AMV
8	-	-	69	AZT, ddI, ddC, d4T, 3TC, PFA, ABV, IDV, SQV, AMV

The four known chemotherapeutic HIV protease
inhibitors used for comparative purposes in this example
have been utilized in actual human HIV chemotherapy, and
are: Ritonavir ("RTV," Abbott Laboratories); Indinavir

5   ("IDV," Merck Research Laboratories); Amprenavir (AMV,
See Ghosh et al., *Bioorg. Med. Chem. Lett.*, 8, 687-690
(1998)); and Saquinavir ("SAQ", Roche Research Centre).
The IC_{50} values (μM) for all five compounds were
determined with respect to wild-type and multidrug-

10   resistant HIV-1.

      To determine protease inhibitory activity against
multidrug resistant HIV, the IC_{50}'s were measured against
a panel of clinically isolated mutant HIV isolates. The
IC_{50}'s were determined by utilizing the PHA-PBMC exposed

15   to HIV-1 (50 $TCID_{50}$ dose/1X10^6 PBMC) as target cells and
using the inhibition of p24 Gag protein production as an
endpoint.

      The IC_{50}'s were determined by utilizing the PHA-PBMC
assay in which target cells are exposed to HIV-1 (50

20   $TCID_{50}$ dose/1X10^6 PBMC) and inhibition of p24 Gag protein
production is used as an endpoint. All drug
sensitivities were performed in triplicate. In order to
determine whether the HIV isolates were syncitium
inducing (SI) or non-syncitium inducing (NSI), an aliquot

25   of viral stock supernatant, containing 100 $TCID_{50}$, was
cultured with 1 X 10^5 MT-2 cells in a 12-well plate.
Cultures were maintained for four weeks and were examined
for syncytium formation twice a week. The results are
shown below in Table 3.

30

79

Table 3

IC$_{50}$ (μM)						
Pheno-type	Patient/Isolate code (See Table 2)	RTV	IDV	AMV	SAQ	Compound 32
SI	HIV-1$_{BRS104pre}$	0.055	0.013	0.021	0.01	<0.001
SI	HIV-1$_{LAI}$	0.0047	0.019	0.019	0.0054	0.0004
NSI	HIV-1$_{BAL}$	0.018	0.0056	0.014	0.0037	0.0004
	1	>1	>1	0.29	0.29	0.002
	2	>1	0.24	0.24	0.035	<0.001
	3	>1	0.46	0.33	0.036	<0.001
	4	>1	0.24	0.4	0.033	0.001
NS1	5	>1	0.8	0.28	0.24	0.002
	6	>1	0.37	0.11	0.19	<0.001
	7	>1	>1	0.42	0.12	0.004
	8	>1	>1	0.22	0.009	0.001
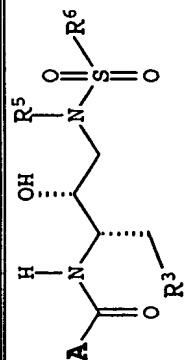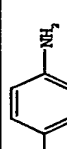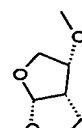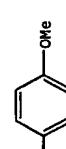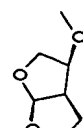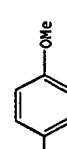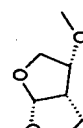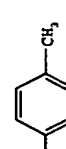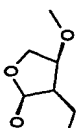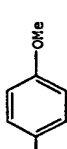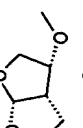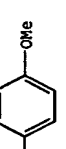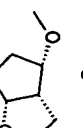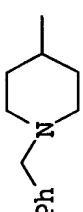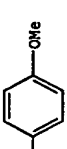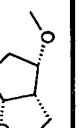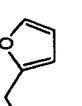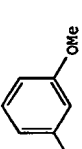
　　　The above IC$_{50}$'s clearly demonstrate the broad-
spectrum and extraordinarily potent activity of compound
5　32 against wild-type HIV-1 and the eight different
multidrug-resistant clinical isolates tested as was
predicted from the biochemical fitness profiles in Example
13. For example, compound 32 exhibits nanomolar and sub-
nanomolar potency against all the multidrug-resistant
10　strains tested, whereas Ritonavir, a reasonably potent
wild-type inhibitor, is virtually inactive toward the
resistant viruses. Moreover, compound 32 is about 9 to
about 150 times more potent against the multidrug-
resistant viruses than Saquinavir, one of the most potent
15　known compounds against known multidrug-resistant strains
of HIV-1. Patients with viral plasma loads greater than
10,000 RNA copies/mm^3 are at risk for developing fatal
AIDS complications. There are no effective therapeutic
options currently available for these patients infected
20　with these multidrug resistant viruses. Compound 32 and
analogs thereof are predicted to be potent in preventing
the selection of these viral strains in vivo.

Example 15

This example demonstrates the wild-type antiretroviral activity of the compounds of the present
5   invention.

It is predicted that the activity of the present inventive compounds against wild-type HIV protease correlates with of antiretroviral activity against multidrug-resistant HIV.   Numerous compounds of the
10   present invention were tested against wild-type HIV (See,, Ghosh et al., *J. Bioorg. Med. Chem. Lett., 8,* 6870690 (1998)).   Exemplary compounds, which demonstrate potent wild-type HIV protease activity, are shown below in Table 4.

Table 4

Structure common to Table 4 (A–C(=O)–NH–CH(CH$_2$R^3)–CH(OH)–CH$_2$–N(R^5)–SO$_2$R^6)

A	R^3	R^5	R^6	Ki (nM)	ID$_{50}$ (nM)	Comments
bicyclic (OMe)	Ph	isobutyl	4-NH$_2$-phenyl	2.1	4.5	Compound 32 (Fig. 3A)
bicyclic (OMe)	Ph	isobutyl	4-OMe-phenyl	1.1	1.4	Compound 34 (Fig. 3B)
bicyclic (OMe)	Ph	isobutyl	4-OMe-phenyl			
bicyclic (OMe)	Ph	isobutyl	4-CH$_3$-phenyl	1.2	3.5	
bicyclic (OMe)	Ph	isobutyl	4-OMe-phenyl	2.2	4.5	
bicyclic (OMe)	Ph	isobutyl	4-OMe-phenyl			
bicyclic (OMe)	4-methyl-1-benzyl-piperidinyl	isobutyl	4-OMe-phenyl			
bicyclic (OMe)	Ph	2-furylethyl	3-OMe-phenyl			

It is believed that the above compounds in Table 4 will
prevent the emergence of resistance in an HIV-infected human.

Example 16

5   This example demonstrates the oral absorption of compound
32 in an *in vivo* experimental model.

        Compound 32 was orally administered to a rat at a dose of
about 40 mg per kg body mass, using a PEG 300 vehicle as a
carrier.   The plasma blood levels of compound 32 were measured
10   over a 24 h period after oral administration.   The results are
shown in Table 5 below.

Table 5

Time After Administration		Plasma Concentration	
Hours	Minutes	(nM)	(ng/mL)
0.28	17	1598	898
1.00	60	878	493
2.07	124	626	352
4.01	240	670	377
6.01	360	594	334
8.05	483	1115	627
12.04	722	246	138
14.08	845	102	57
24.00	1440	82	46

15   These results demonstrate that compound 32 maintains high
blood levels (e.g., nearly 0.6 uM after 6 hours) long after
oral administration. Although applicants do not wish to abound
by any one particular theory, it is believed that the non-
peptide structure of the compounds of the present invention
20   make them less prone to biological (e.g., enzymatic)
degradation, and thereby contribute to their prolonged blood
levels after oral administration.   From these data, the
compounds of the present invention are predicted to have

excellent oral bioavailability in humans, and maintain
therapeutically significant blood levels over prolonged periods
after oral administration.

5   Example 17

         This example demonstrates the influence of human protein
binding on the antiviral activity of compound 32.   Several
potent and orally bioavailable HIV protease inhibitors failed
to have in vivo antiviral efficacy.   These failures have been
10   ascribed, but not definitively proven, to be due to excessive
binding to human plasma proteins, particularly serum albumin
and AAG.   The protein binding against human alpha acid
glycoprotein (AAG, 10 µM) and against human serum albumin (HAS,
300 µM) were compared for compound 32 and amprenavir, a
15   structurally related analog that is an FDA approved drug.   The
results are shown in Table 6.

Table 6

Compound	IC_{50} (µM)		
	(-)	AAG	Alb
32	0.0015 (1X)	0.0022 (1.5X)	0.003 (2X)
amprenavir	0.029 (1X)	0.18 (6X)	0.021 (1X)

         These data demonstrate that the presence of AAG and HAS in
20   physiologically excessive amounts does not adversely affect the
antiviral activity of compound 32.   From these data, the
affinity of compound 32 for human AAG and HSA is predicted to
be actually lower than that for amprenavir, a known drug.   From
these data, the compounds of the present invention are expected
25   to have excellent in vivo efficacy in humans, and maintain
therapeutically significant levels over prolonged periods of
time.

Example 18

    This example describes the inhibitory activity of compounds
35 (Fig. 5A), 36 (Fig. 5B), 37 (Fig. 5C) and 38 (Fig. 5D). In
accordance with the technique disclosed in Example 13 above, the
5 inhibitory activity of these compounds was tested against
proteases of the wild-type HIV-1. Compound 36, 37 and 38 were
also tested against proteases containing the deleterious drug
resistance associated mutations V82F/I84V and G48V/V82A. Fitness
was determined in accordance with Example 13. The results of
10 these experiments are shown below in Table 7.

Table 7

COMPOUND	ENZYME	K_i (pM)	K_{I-wt}/K_{I-mut}	Fitness
35	WT	81	1	
36	WT	5<		
	V82F/I84V	24.4	>4.9	>0.8
	G48V/V82A	15.3	>3.0	>0.8
37	WT	12	1	
	V82F/I84V	25.7	2.1	0.3
	G48V/V82A	64	5.3	1.4
38	WT	>5		
	V82F/I84V	66.8	>13	>2.1
	G84V/V82A	34	> 6.8	>1.8

15   These results further demonstrate compounds of the present
invention that are potent inhibitors against mutant proteases.
Based on the fitness profile, it is believed that drug
resistant viruses containing the characteristic mutations
assayed herein will not emerge from the wild-type in the
20 presence of compound 37.

Example 19

    This example further demonstrates the broad-spectrum and
potent activity of exemplary compounds of the present invention
25 against multidrug-resistant clinical isolates.

The IC_{50} values (μM) for all compounds 32, 35, 36, 37, and 38 were determined with respect to wild type clinical isolates HIV-1_{LAI} and HIV-1_{BaL}. The latter is a monocytotropic strain of HIV.

The IC_{50}'s for isolates HIV-1_{LAI} and HIV-1_{Ba-L} were determined by exposing the PHA-simulated PBMC to HIV-1 (50 $TCID_{50}$ dose/1X10^6 PBMC), in the precence of various concentrations of compounds 32, 35, 36, 37 and 38, and using the inhibition of p24 Gag protein production as an endpoint on day 7 of culture ("p24 assay"). All drug sensitivities were performed in triplicate. The IC_{50}'s for isolate HIV-1_{LAI} were also determined by exposing MT-2 cells (2x10^3) to 100 $TCID_{50}$s of HIV-1_{LAI} cultured in the presence of various concentrations of compounds 32, 35, 36, 37 and 38. The IC_{50}'s were determined using the MTT assay on day 7 of culture. All sensitivities were determined in duplicate. The results are shown below in Table 8.

Table 8

Virus	Cell Type /Assay	Comp. 32 IC_{50}(μM)	Comp. 35 IC_{50}(μM)	Comp. 36 IC_{50}(μM)	Comp. 37 IC_{50}(μM)	Comp. 38 IC_{50}(μM)
HIV-1_{LAI}	MT-2/MTT	0.00022	0.028	0.017	0.0053	0.028
HIV-1_{LAI}	PBMC/p24	0.00022	0.020	0.034	0.0027	0.0080
HIV-1_{Ba-L}	PBMC/p24	0.00033	0.013	0.038	0.0030	0.0093

These results demonstrate the potent antiretroviral activity of particular compounds of the present invention.

Example 20

This example further illustrates the potent and broad-spectrum multidrug-resistant antiretroviral activity of an exemplary compound of the present invention.

5   Compound 32, shown in Figure 3A, was tested against various mutant multidrug-resistant HIV-1 strains clinically isolated from patients. These isolates were all taken from patients who failed therapy on one or more HIV protease inhibitors due to high level clinical resistance. All of these isolates exhibit

10   high level phenotypic resistance in antiviral assays against many of the commonly use HIV protease inhibitor drugs. Compound 32 was tested against these multidrug-resistant clinical isolates side-by-side with known drugs that are commonly used in HIV antiviral therapy, including reverse transcriptase

15   inhibitors such as AZT, 3TC, DDI, DDC, and D4T, and protease inhibitors such as Indinavir (Ind.), Nelfinavir (Nel.), Ritonavir (Rit.), and Saquinavir (Saq.). The IC_{50}'s for compound 32 and the comparative drugs against the multidrug-resistant HIV-1 clinical isolates, and against wild-type HIV-1 (WT), are

20   shown in Table 9a.

The mutant multidrug-resistant HIV-1 strains corresponding to each patient, numbered 9-35, were genetically analyzed in terms of the nucleic acid sequences of the protease (PR) and a portion of the reverse transcriptase (RT) genes from which

25   mutations in these enzymes were determined. The mutations in the protease and reverse transcriptase of the multidrug-resistant viruses isolated from each patient are shown below in Table 9b.

Table 9a

Patient Isolate	AZT	3TC	DDI	DDC	D4T	Ind.	Nel.	Rit.	Saq.	Comp. 32
					IC$_{50}$ (µM)					
9	0.01	0.39	0.7	0.15	0.91	1.087	0.98	0.53	>0.3125	0.0003
10	0.02	1.35	1.7	0.37	1.29	>1.25	>1.25	2.03	>0.3125	0.0017
11	0.11	23.61	2.4	0.18	3.10	0.012	0.03	0.01	0.001	0.0004
12	0.07	0.78	0.9	0.20	1.23	>1.25	>1.25	2.47	>0.3125	0.0010
13	0.17	1.04	0.5	<0.1221	0.78	>1.25	0.47	1.64	>0.3125	0.0004
14	0.64		2.4	<0.1221	1.10	0.089	0.01	0.04	0.040	0.0003
15	0.20	>31.25	2.2	0.32	1.10	0.265	0.47	1.14	>0.3125	0.0011
16	0.97	27.98	3.5	0.57	1.81	0.384	0.86	1.34	>0.3125	0.0031
17	>1.25	28.05		0.63	4.28	0.502	0.52	0.87	0.107	0.0022
18	0.55	>31.25	2.2	0.48	2.08	0.369	0.60	3.02	0.039	0.0019
19	>1.25	>31.25	36.6	6.80	35.63	0.784	0.50	2.94	0.055	0.0005
20	1.25	3.21	7.1	0.57	22.54	0.591	0.58	1.90	0.032	
21	>1.25	1.69	1	0.38	3.28	1.250	>1.25	2.18	0.21	0.0023
22	1.02	>31.25	3.7	0.63	4.68	0.173	0.10	0.56	0.003	
23	0.19	>31.25	1.8	0.28	1.00	0.461	0.28	1.82	0.008	0.0004
24										0.0004
25										0.0019
26										0.0019
27	0.03	1.72	2.6	0.41	4.00	>1.25	>1.25	2.97	>0.3125	0.0009
28	>1.25	2.08	2.8	0.36	5.44	1.040	>1.25	2.66	>0.3125	
29	>1.25	2.24	3.8	0.34	5.29	0.569	0.67	0.36	0.050	0.0009
30	0.16	>31.25	2.8	0.24	2.52	0.270	0.52	1.03	0.191	0.0019
31		>31.25	2.6	<0.1221	3.11	0.251	0.24	0.85	0.074	0.0010
32	0.32	>31.25	8.4	0.91	2.41	0.223	0.22	0.37	>0.3125	
33	0.51	>31.25	2.0	0.28	2.73	0.133	0.35	0.18	0.059	0.0005
34	>1.25	>31.25	9.1	1.13	7.71	0.595	0.26	3.38	0.063	0.0024
35	0.88	>31.25	17.0	2.46	18.13	0.509	0.48	2.60	0.0616	0.0012
(WT)	0.022	0.264	0.895	0.243	1.059	0.02	0.031	0.019	0.007	0.0007

Table 9b

Isolate		Mutations															
9	PR	V003I	L010I	S037N	R041K	G048V	I054S	I062V	L063S	1064L	A071V	V082A	1093L			
	RT	P004S	V060I	V090I	E122K	E194E/K	Q174K	G196E	R211K	L214F	V245M	R227K	E297R	L301L/I		
10	PR	V003I	L010I	S037N	R041K	G048V	I054S	I062V	L063S	1064L	A071V	V082A	1093L			
	RT	P004S	V060I	V090I	E122K	T165A/T	Q174K	Y181C	E194K	G196E	R211K	L214F	H221H/Y	V245M	R277K	
11	PR	V003I	L010I	1015V	M036I	S037N	R041K	L063T	1093L							
	RT	K020R/K	M041L	K043Q	E044D	V060I	D067N	T069D	E122E/K	D123E	Y181C/Y	M184V	G196E	H208Y	L210W	R211K
12	PR	V003I	L010I	1015V	M036I	S037N	R041K	G048V	I054T/I	L063T	A071V	T074A	V082A/V	1093L		
	RT	M041L	K043Q	E044D	V060I	D067N	T069D	L074L/I	K103N	D123E	I135T	Y181C	G196E	H208Y	L210W	R211K
13	PR	V003I	L010I	1015V	M036I	S037N	R041K	G048V/G	I054T/I	Q058E/Q	Q061R/Q	L063T	A071A/V	1072T/I	V082A	1093L
	RT	M041L	K043Q	E044D	V060I	D067N	T069D	L074L/I	K103N	D123E	I135T/I	Y181C	G196E	H208Y	L210W	R211K

(Table 9b con't.)

14	PR	V003I	L010I	K020R	E035D	M036I	S037D	R041K	G048V	L063C	A071V	1072T	V082A/V	1093L
	RT	M041L	L074L/V	T069T/N	E122K	D123E	Y181C	Q207E	L210W	R211K	L214F	T215Y	L228R	E248D
		R277K	E297K											
15	PR	V003I	L010I	E035D	R041K	L063P	A071A/V	I072V/I	G073R/C	V077I	1084V	L090M	1093L	
	RT	D067N	T069D	I142V	E169D	Y181C	M184V	Q207B	R211K	L214F	T215Y	D250E	P272A	Q278E
		L283I	I293V											
16	PR	V003I	L010I	1013V	E035D	S037A	R041K	L063P	A071V	G073S	I084V	L090M		
	RT	K020R	M041L	K043N	D067N	D123N	I178M/I	M184V	G196E	E203D	L214F	T215Y	K219Q	
		R277K	G333E	A360T										
17	PR	V003I	L010I	1013V	E035D	S037A	R041K	L063P	A071V	G073G/S	I084V	L090M		
	RT	K020R	M041L	K043N	D067N	D123N	I178M/I	M184V	G196E	E203D	L214F	T215Y	R277K	
		G333E												
18	PR	V003I	L010V	S037N	K043T	I054V	L063P	A071V	L090M					
	RT	K020R	V035M	K064H	D067G	T069N	K070R	K102R/K	V1118I	E122K	I135T	S162A	M184V	T215S
		D128E	K219Q											
19	PR	V003I	L010I	S037Q	M046L	I054V	R057K	L063P	A071V	V082A	L090M			
	RT	K020R	T058N	S068G	T069T/I	V075I	F077L	A098S	K103N	F116Y	I135T	I142M	Q151M	
		Y181C	M184V											

(Table 9B con't.)

#	Type	Mutations
20	PR	V003I, L010I, T012P, K014R, I015V/I, G016E, S037N, M046I, I054V, K055R, I062V, L063N, A071T, V077I, V082A, I085V, L090M
	RT	K020R, V035I, T039A, M041L, K043E, E044A, D067N, V075A, K103N, V118I, I135M, Y181C, H208Y, L210W, R211K
21	PR	V003I, L010I, I015V, K020R, E035D, M036I, S037K, R041N, K043T/K, M041I, L063P, H069K, A071V, T074S, V082F, N088E, L084M, L090M, I093L
	RT	K020R, V035T, T039R, M041L, K043E, E044D, V060I, I063M/I, D067N, T069D, A098G, V118I, D121H, I135T/I, I142V
22	PR	V003I, L010I, E034E/Q, S037H, M046I, I054V, I062V, L063S, V082A, L089L/M
	RT	K020R/K, T039A/T, M041L, K043E, E044D, D067N, V118I, M184V, E203E/K, Q207E, H208Y, L210W, R211K, L214F, T215Y
23	PR	V003I, L010I, I015V, K020I, L024I, M036I, S037N, I054V, R057K, L063P, A071V, V082A
	RT	K011R, D067N, K070R, I135T, Y181V/D, M184V, D218E/D, K219Q, P272A, R277K, R284R/K, I293V, E297V, M357T/M, G359G/S
24	PR	V003I, I015V, D030N, E035D, S037D, L063P, V077I, N088D
	RT	K064R, E122K, D177E, M184V, G196R, R211G, L214F, V245T/M, E297A, N348I, R358K
25	PR	V003I, K020I, T026T/I, S037N, M046I, L063P, A071V, G073S, V077I, I084V, L090M, I093L
	RT	V035M, D067N, T069D, K070R, D177E, E122P, M184V, I202V, Q207E, R211K, L214F, T215F, K219Q, E224K, R277K

(Table 9b con't.)

26	PR	V003I	L010I	S037N	R041K	G048V	I054S	I062V	L063S	I064L	A071V	V082A	I093L
	RT	P004S	V060I	V090I	I135Y	T135A/T	Q174K	Y181C	E194K	G196E	R211K	L214F	H221H/Y
		V245M	R277K										

27	PR	V003I	L010I	K020R	S037N	M036I	R041K	G048V	I054T/I	L063T	A071A/V	T074A	V082A
		I093L											
	RT	M041L	K043Q	E044D	V060I	D067N	T069D	L074L/I	K103N	F116F/L	D123E	Y181C	G196E
		H208Y	L210W										

28	PR	V003I	L010I	I015V	M036I	S037D	G048V	I054V	D060E	Q061E	I062V	I064V	A071V	V082A
		L090M	I093L											
	RT	P004S	M041L	D067N	T069D	K070R	V090I	K103N	I135T	S162A	V179I	Y181C	G196E	Q207E
		L214F	T215F											

29	PR	V003I	L010I	K020I	S037N	M046M/I	L063P	I072I/K	G073C	V077I	L090M
	RT	V035I	T039A/E	M041L	E044D	L074L/V	R083K	K102Q	S162C	I178L	E203K	H208Y	L210W	R211K
		L214F	T215Y											

30	PR	V003I	L010I	E035D	R041K	L063P	A071A/V	I072V/I	G073G/S	V077I	I084V/I	L090M	I093L
	RT	D067N	T069D	I142V	E169D	Y181C	M184V	Q207E	R211K	L214F	T215Y	D250E	P272A	Q278E
		L283I	I293V											

(Table 9b con't.)

		Mutations
31	PR	V003I L010L/I E035D M036M/I S037N M046X I054V L063P I066F A071V V082A/T I084V/I
	RT	K032R/K K064R D067N K070R K103N/K E122K R211K Y181F/C M184V L214F D218E K219Q E248D T286A I293V
32	PR	V003I L010I S037N G048V I054V I062V/I L063P V077I V082A I093L
	RT	K020R M041L D123N I178L M184V T200A/T A071A/T L210L/W L214F T215Y R277K T286A Q334L/Q T338S/T
33	PR	V003I L010I E035D M036I S037D D060E L063P I064V L090M
	RT	M041L/M D067N T063T/N K070R D177D/E M184V I202V Q207E L210W R211K L214F T215Y K219Q V245T P272A
34	PR	V003I L010V S037N K043T I054V L063P A071V V082A L090M
	RT	K020R V035M K064H D067G T069N K070R K102R/K V118I E122K I135T S162A M184V T215S D218E K219Q
35	PR	V003I L010I L019I S037Q M046L I054V R057K L063P A071V V082A L090M
	RT	K020R T058N A062V S068G T069T/I V075I F077L A098S K103N F116Y I135T I142M Q151M Y181C M184V

The results of this experiment further show the
effectiveness of an exemplary compound of the present
invention against a wide range of viral mutants compared to

5   other well-known inhibitors. These mutant viruses represent
a panel of the most broadly cross resistant clinical
isolates known to date based on their resistance to
therapeutically used HIV protease inhibitors. Compound 32
was consistently potent against all of the clinically

10   isolated mutant viruses tested, and was significantly more
potent against these multidrug resistant viruses than the
comparative drugs which are currently used in human HIV-1
therapy. Compound 32 was ten to one-thousand times more
potent against these viruses than even saquinavir, one of

15   the most potent known compounds against multidrug-resistant
HIV-1. Based on the high potency, it is believed that these
mutants will not only be inhibited, but also that these
mutants would not be able to emerge if the compound is
administered to a patient infected with a predecessor virus.

20

All of the references cited herein, including
patents, patent applications, and publications, are hereby
incorporated in their entireties by reference.

25   While this invention has been described with an
emphasis upon preferred embodiments, it will be obvious to
those of ordinary skill in the art that variations of the
preferred embodiments may be used and that it is intended
that the invention may be practiced otherwise than as

30   specifically described herein. Accordingly, this
invention includes all modifications encompassed within

the spirit and scope of the invention as defined by the following claims.